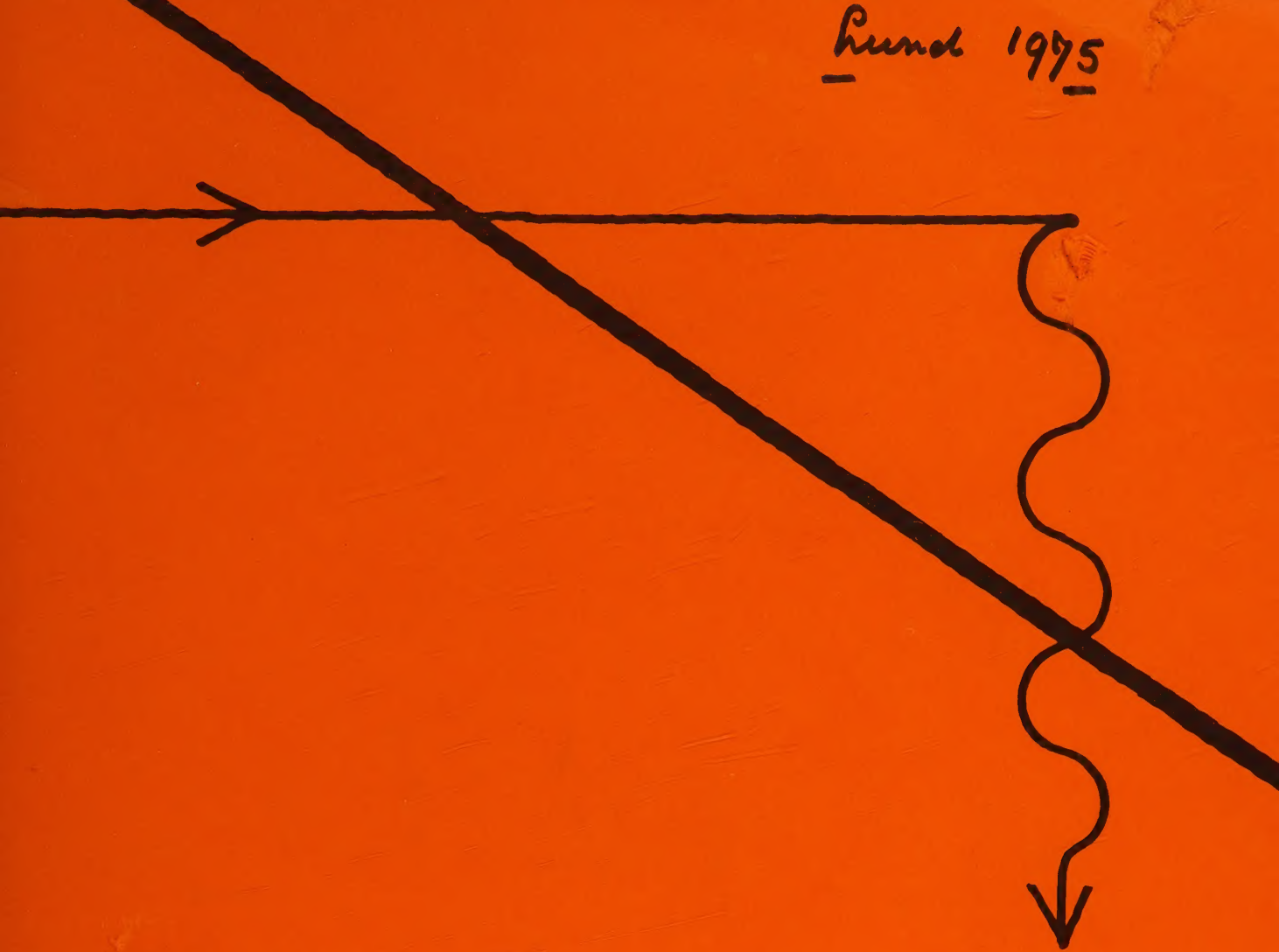


Rund 1975



TRACE ANALYSIS
OF THICK SAMPLES
BY PROTON-INDUCED
X-RAY EMISSION

Mats Ahlberg

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av

Mats Ahlberg

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Akademisk avhandling som för avläggande av teknisk doktorsexamen vid tekniska fakulteten vid universitetet i Lund kommer att offentligen försvaras å föreläsningssal B, fysiska institutionen i Lund, onsdagen den 3 december 1975 kl 10.15.



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SUMMARY

Introduction

Trace elements are often of fundamental importance in science, medicine and technology (1, 2, 3). In physical science, their presence has an important influence on a variety of electrical, optical and magnetic properties. In biology, for example, traces of metals are essential for a healthy life. In addition, the increasing pollution of air and water has made it important to study the toxic trace elements, like, for example, the heavy metals. The mechanism of action of many of the trace elements is still largely unknown.

Considering the number of trace elements often present in a given system, multi-element analytical methods applicable to a wide range of samples from biology, environment and technology are required for the study of their role. For survey purposes, methods which do not require predetermination of the elements to be analysed and which have a sensitivity that varies only slightly with atomic number are of special interest. Analytical techniques using X-ray emission analysis are examples of such methods.

In 1970, Johansson et al (4) introduced Proton-Induced X-ray Emission (PIXE) in trace analysis, employing protons in the MeV range for the excitation and solid state detectors for the detection of the characteristic X-rays. By now, PIXE has developed into a reliable tool for the routine multi-element trace analysis of thin samples (5).

The present work extends PIXE to the trace analysis of thick samples, i.e. when X-ray absorption and the decrease in X-ray production cross section due to the slowing-down of the protons in the sample have to be accounted for. The technique for the analysis of electrically-conducting samples of heavy elements and of insulators is outlined. The possibility of obtaining a

simple depth distribution from one irradiation of the sample is examined and an application of a method for quantitative determination of light elements beyond the scope of X-ray analysis is shown.

Characteristic X-rays or Auger-electrons are emitted when vacancies in inner electron shells in atoms are filled with electrons from outer shells. The energies of the X-rays and of the Auger-electrons are characteristic for each element and their intensities depend on the amount of the element present and the rate in which vacancies are produced. Thus, qualitative analysis can be performed by measuring the energy of the characteristic X-rays and quantitative analysis by measuring the number of X-rays emitted from a certain element, knowing the probability of inducing a characteristic X-ray. This probability depends on the kind of radiation used for excitation and is proportional to the intensity of the exciting radiation

Irradiation of the sample with X-rays or electrons is the most common mode of excitation but the sensitivity is limited by the background radiation which consists of scattered X-rays or bremsstrahlung. The background of bremsstrahlung associated with irradiation with charged particles is comparatively low and as the X-ray production cross sections for protons of some MeV are comparable with those for electrons and X-rays in the keV range, the signal-to-background ratio for proton-induced X-ray emission is very favorable. Furthermore, intense, well-focussed proton beams are available from, for example, Van de Graaff accelerators, allowing very small samples to be analysed with a high sensitivity.

Proton-Induced X-ray Emission

In PIXE analysis, the sample is irradiated with protons and the characteristic X-rays emitted are detected by a semiconductor detector coupled to a multichannel analyser or to a small computer. Typical parameter ranges are proton energies of 1 - 5 MeV, beam currents 1 - 1000 nA and analysing times 100-1000 seconds. Detection limits down to 10^{-12} g are obtained. Quantitative ana-

lysis is generally performed for elements with atomic number $Z \geq 15$. K X-rays are normally used for the elements up to $Z \approx 55$, while for heavier elements, L X-rays are used. The characteristic X-rays from elements lighter than phosphorus are lower than 2 keV and uncertainty in the calculation of the absorption of such low energy X-rays usually makes only qualitative analysis possible. PIXE is capable of the simultaneous analysis of 10 - 20 elements with a detection limit which varies only slightly with the atomic number of the element. The procedure is fast and cheap, especially when compared with single element analytical methods. PIXE is especially advantageous if large samples are expensive or impossible to obtain. If the samples are thin ($\leq 1 \text{ mg/cm}^2$), no correction for X-ray absorption or proton energy loss in the sample is necessary.

An example of an ideal situation for PIXE analysis is that described in Paper I. Coloured marks and shades were found on "pendants" of black slate from the late Neolithic of south Scandinavia and a rapid determination of their composition appeared to be desirable. A survey analysis with a broad proton beam showed traces of gold and silver on two of the "pendants" and in a subsequent scanning of these two with a narrower beam, it was possible to distinguish between three different gold-silver alloys. The calculated gold-silver layer was always less than $25 \text{ } \mu\text{g/cm}^2$. Thus it could be treated as a thin sample although it was situated on a thick backing.

Analysis of Thick Samples

Very often analysis of thick samples are to be preferred. It can be the case that e.g. thin samples are hard or tedious to prepare or that the risk of contamination or destruction of the sample makes analysis of a thick sample desirable. When irradiating thick samples, the continuous background radiation of scattered X-rays and bremsstrahlung from protons and secondary electrons becomes more intense. The yields of characteristic X-rays are also increased.

In trace analysis, the trace elements to be determined exist in a so-called "matrix" which consists of the major constituents of the sample. To account for X-ray absorption and the decrease in cross section due to the slowing-down of the protons in the sample, the mass absorption coefficient and the stopping power of this matrix have to be known. The composition of the matrix is almost always known well enough for the stopping power and mass absorption coefficient to be accurately calculated as weighted averages of the values for the major elements. Values of stopping power and mass absorption coefficients for the elements are available (6, 7). This approach to the trace analysis of thick samples makes it possible to analyse every kind of sample and is used for black slate in Paper I, two different specimens of steel in Paper II, tooth enamel and dentine in Paper IV and aluminum in Paper V. One must, however, remember that we have assumed a homogeneous sample and that only a surface layer is analysed. The thickness of the analysed layer varies with the matrix, with X-ray and proton energies and, if the trace elements have a depth profile within this layer, uncertainties in the calculated concentrations are introduced.

Detection limits

The lower limit of detection is an essential factor in trace analysis and must be evaluated separately for every single case. The detection limit of an element is here defined as the concentration of that element which gives rise to $3\sqrt{B}$ counts in a peak, where B is the number of background pulses in an interval of 2 FWHM (full width at half maximum peak-height) around the peak energy. By using this criteria, the detection limits derived for elements with $Z = 15 - 92$ in steel specimens (Paper II) and in tooth materials (Paper IV), can easily be converted to apply to other counting rates and analysing times than those used. As the counting rate in the detector is limited by the electronics available, the sensitivity depends on the efficiency with which we can use the capacity of the detector. By employing absorbers between the sample and the detector, we can reduce the intensity of the background radiation. For elements with characteristic X-rays of high energies, low detection limits are obtained by using high proton energies and thick, low Z absorbers to reduce the intense low energy brems-

strahlung, which comes mainly from secondary electrons. As the maximum energy of the bremsstrahlung from secondary electrons depends on proton energy, a thinner absorber and a lower proton energy is a more favorable combination for elements with characteristic X-rays of low energies. Problems arise when the matrix consists of heavy elements which give a significant contribution of characteristic X-rays to the counting rate. By using absorbers with an absorption edge just below that of the characteristic X-rays from a major element, the intensity can be reduced while the transmission for lower energy X-rays is still large. In Paper II, detection limits around 10 ppm (parts per million) are obtained even below the intense Fe K_{α} peak from the steel specimen by using a chromium absorber.

Due to interference with characteristic X-ray peaks from other elements present in the sample, some elements may not be determined by their most intense X-ray line. Also, it is notable that the detection limits, when using thick absorbers, may be lower for L_{β} X-rays than for L_{α} X-rays although the primary yield of the former is only about 0.6 times that of the latter. This is because L_{β} X-rays have a higher energy and consequently better transmission out of the sample than the corresponding L_{α} X-rays and also that the L_{β} X-rays are often situated on a lower continuum than the L_{α} X-rays due to the energy dependence of the bremsstrahlung radiation.

Charging

When an insulating sample is bombarded with protons in vacuum, it becomes highly charged. In the discharge, electrons are accelerated and produce bremsstrahlung of several keV. This bremsstrahlung reduces drastically the sensitivity both due to the higher background level and also to the fact that the proton current has to be decreased to adjust for the higher counting rate in the detector. Paper III describes two ways of neutralizing the charge build-up without contamination of the sample; by the insertion of a hot carbon filament, emitting electrons, a short distance in front of the sample and by raising the pressure in the target chamber.

Enhancement effects

Enhancement effects, i.e. secondary production of characteristic X-rays by proton induced characteristic X-rays, secondary electrons and bremsstrahlung cannot always be neglected in quantitative PIXE analyses. In Paper IV, it is shown that the first of these effects gives a significant contribution to the X-ray yield from an element only when the absorption edge energy of the element is immediately below an intense X-ray line from a major element in the matrix. This is due to the fact that the cross section for proton induced X-ray production is a decreasing function of absorption edge energy while the cross section for X-ray induced X-ray production is an increasing function of the same parameter as long as the energy of the primary X-rays is higher than that of the absorption edges. Secondary production from secondary electrons and bremsstrahlung are negligible. In an enamel matrix, we get significant enhancement effects for the elements with absorption edges below 3.69 keV, the Ca K_{α} X-ray energy. For 2 MeV protons the enhancement from 40% calcium in an enamel matrix is 15% for K K_{α} and for P K_{α} it is decreased to 1.3%.

Surface roughness

In the calculations of the X-ray yield from thick samples a flat surface is assumed. In many situations, this is not a very good assumption. In Paper IV, the effect on the X-ray yield from a tooth matrix when the surface is approximated by a sawtooth function with a peak angle of 90° is calculated for 2.5 MeV protons. Height differences corresponding to 9 mg/cm^2 decrease the X-ray yield as much as 30% for X-rays between 2 and 4 keV. For X-rays of higher energies, the decrease is less, being above 8 keV below 10%. With a height difference of 4 mg/cm^2 , the decrease in the X-ray yield is less than 5% for all characteristic X-ray energies above 2 keV.

Depth profiles

In the analysis of thick samples, it is often important to be able to distinguish whether an element is situated on the surface of the sample or distributed within it. In Paper V, it is shown that

there is a possibility of determining simultaneously both the surface concentration and the concentration within the sample of an element, by studying both the α and β X-rays of the element. Due to the energy dependence of the mass absorption coefficient, α X-rays are more absorbed in the sample than corresponding β X-rays.

Therefore the β/α ratio for an element is larger when the element is distributed within the sample than when it is situated on the surface. The results for iron in an aluminum matrix are compared with those obtained by making irradiations with two different proton energies or two different target angles. The method of using two different target angles is less precise than the method of measuring both the α and β X-rays simultaneously. The best precision is obtained using two different proton energies, but this means that two irradiations have to be made.

Light elements

Due to the strong absorption of the weak characteristic X-rays from lighter elements, quantitative PIXE analysis is usually not advisable for elements lighter than phosphorus. For thin samples, a suitable method to use instead of PIXE is Rutherford backscattering (8). For thick samples, however, Rutherford backscattering is not so suitable since there will be a high background of scattered protons which have lost some of their energy when passing through the sample. As the Coulomb barrier is low for light elements, nuclear reactions with low energy protons are possible. Due to their large Q-values, such reactions often result in charged particles and high energy gamma rays. Contrary to X-ray excitation methods, nuclear reactions often exhibit sharp resonances, which allow depth profile measurements. In Paper VI, the nuclear reactions $^{19}\text{F}(p,\alpha\gamma)^{16}\text{O}$ and $^{15}\text{N}(p,\alpha\gamma)^{12}\text{C}$ are used for the study of fluorine and nitrogen concentrations in developing dental enamel. The nitrogen concentration permits an estimation of the protein content which is important since this is a well-known function of enamel development. By using the two resonances at 872 keV and 935 keV, respectively, for the fluorine analysis and the resonance at 897 keV for the nitrogen determination, results of the fluorine and

the nitrogen concentrations are always obtained from the same depth region. The lowest quantity of fluorine detected was 2×10^{-13} g, which can be regarded as about the limit of detection. As the sensitivity and the proton energies used are in the same region as in PIXE analyses, proton activation analysis is a very good complement to PIXE for the analysis of light elements when suitable nuclear reactions exist.

Accuracy and Precision

To evaluate the accuracy and the precision of PIXE when applied to thick samples, several tests have been performed. In Paper IV, the analysis of a fluorapatite standard with known concentrations of major elements from wet chemical analysis is performed. In addition, some results from an intercomparison study (9) are presented. One of the samples analysed was a Millipore cellulose membrane filter in which the elements Al, S, K, V, Cr, Mn, Fe, Zn, Cd and Au were deposited. The maximum correction factor to a thin sample as calculated by the equation for thick samples was 3.3 for sulphur. The main interferences to account for were Au M + S K, Cd L + K K, V K_{β} + Cr K_{α} , Cr K_{β} + Mn K_{α} , Mn K_{β} + Fe K_{α} and Zn K_{β} + Au L_{α} .

The results indicate that the precision of PIXE when applied to thick, flat samples can be pressed to 5 %. Due to errors in the mass absorption coefficients, stopping power values and matrix composition the accuracy for a thick, flat sample is about 15 %. Both precision and accuracy are influenced by surface roughness, the effect depending on X-ray energy. Light elements emitting characteristic X-rays of low energies are most affected.

Future of PIXE

The use of PIXE in elemental analysis is growing rapidly all over the world. Particularly for the monitoring of outdoor and indoor air pollution, fast, fully automated techniques for routine analysis are under development. The combination of PIXE with other techniques

used in an accelerator, such as Rutherford backscattering, activation analysis and Auger-electron spectroscopy are probable developments towards a system capable of analysing all elements from hydrogen upwards. Wavelength dispersive crystal spectrometers will be used together with energy dispersive semiconductor detectors for the resolution of interfering X-ray lines and for obtaining information about the chemical state of the element. Well-focussed proton beams have been used for depth profile determinations (10) and the possibility of performing trace analysis of single particles collected from the environment or directly from industrial processes will be of importance for the understanding of the transportation and formation of particulate air and water pollution.

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Acknowledgements

I am deeply grateful to Professor Sven Johansson for stimulating discussions and encouragement and have found my collaboration with Dr Roland Akselsson and Dr Thomas B Johansson most inspiring.

These three dynamic persons, constituting the pioneer PIXE group in Lund, aroused my interest in applications of nuclear physics. Their scientific perspicacity has been of invaluable help for the performance of this work.

I have enjoyed my cooperation with Professor Lars Hammarström and I am grateful to Dr Ragnar Hellborg who taught me, among many other things, the techniques of activation analysis. Each of ten co-authors has contributed to making this work a stimulating and fruitful experience.

Experimental work cannot be performed without skilful technical assistance. Mr Knut Sjöberg has been of a great help in the constructions of sample chambers and the other details needed for sample handling. Mr Kjell Håkansson and Mr Christer Nilsson have always been ready to handle the accelerator, in good spirits even at odd hours. The readiness of everybody who has helped me with shop work, calculations, figures and manuscripts is striking and I owe them my sincere thanks.

The kindness of all my colleagues and friends I have very much appreciated.

GOLDTRACES ON WEDGE-SHAPED ARTEFACTS FROM LATE
NEOLITHIC OF SOUTH SCANDINAVIA ANALYSED BY
PROTON INDUCED X-RAY EMISSION SPECTROSCOPY

M Ahlberg, R Akselsson and B Forkman

Department of Nuclear Physics
Lund Institute of Technology

Lund, Sweden

and

G Rausing

The Historical Museum
University of Lund

Lund, Sweden

ABSTRACT

Visible coloured traces on the surface of two selected wedge-shaped artefacts (pendants) of slate from the late Neolithic of South Scandinavia was analysed by means of proton-induced X-ray emission spectroscopy (PIXE).

PIXE is shown to be a feasible tool in investigating surface layers of archeological significance. Three different gold-silver alloys was found on the two pendants.

The results indicate that we shall have to reconsider the general accepted theories on the economic basis of the early Bronze Age in the area.

PART I

THE WEDGE-SHAPED PENDANTS OF THE SCANDINAVIAN NEOLITHIC

G Rausing

The wedge-shaped "pendants" from the late Neolithic are a type of artefact which up till now has not been ascribed any practical use beyond sometimes being called, presumably incorrectly, whetstones. Their flat sides certainly exhibit grinding marks but instead of being concentrated in the centre, as is the case with recent whetstones, these marks are evenly distributed over the surface, the cross-section of which is either flat or convex, never concave.

These pendants are of several types, the commonest being the wedge-shaped variety with a "hole for a cord" at the thick end. Other forms include miniature axes and dowel-shaped pendants, i.e. pendants with a more or less oval rather than a rectangular cross-section, ending in a point rather than an edge. These deviant types are comparatively rare.

Quite often (45 of the 225 known Scanian specimens, according to Hasselmo*) the wedge-shaped "pendants" bear an engraved decoration on all four sides, though in most cases this has been damaged or partially erased on the lower parts of the flat sides. The pendants are almost all extremely worn at this point but this is not the case with the narrow sides. In the majority of cases most of the wear seems to have occurred more or less parallel to the longitudinal axis of the pendant, though diagonal and transverse marks are also visible in nearly every case.

* Margareta Hasselmo, "Late Neolithic Slate Pendants", paper in comparative Nordic archaeology, Lund 16.12.70 (unpublished, in Swedish)

Compared to the fine polish of the narrow sides, the grooves worn into the flat sides are quite coarse and give the impression of repeated attempts having been made to renew or freshen up the surface and edge of the artefact. With very few exceptions these enigmatic "pendants" have been made from black slate.

Margareta Hasselmo has provided a good survey of the typology of the "pendants", their geographical incidence in southern Sweden and their dating. She also notes that their distribution in Scania seems to coincide with that of the burial mounds of the early Bronze Age, which are confined to a belt roughly 10 km wide along the coast. Strikingly few pendants have been found in definite graves - out of 225 known slate pendants from Scania only 71 were found together with other artefacts, usually in dolmens, passage graves and stone cists, occasionally beneath a mound or stone circle, and of these the position of only 3 could be determined exactly in relation to the skeleton (1 lay above the head, 1 by the breast and 1 beneath the head). Even so, it seems probable that the pendants generally do represent burial finds as do so many other loose finds from the Neolithic and from the Bronze Age, and that when classified as isolated finds, they come from ravaged graves. It also seems likely that the pendants mark the graves of men; where they appear together with other objects the testimony is clear: flint daggers, flint spearheads, flint knives, spoon scrapers, flake scrapers, chisels, saws, flakes and heartshaped and transverse arrow heads of flint, hammer, stones, pottery, arrow shaft polishers, amber buttons, amber rings, a bronze knife, a star-patterned double button of bronze, a reddish glass bead, amber beads, bronze pincers, a bronze rivet and bronze wire.

Clearly the pendants date from the late Neolithic and the

transition to the Bronze Age, the "dagger period". The custom of placing flint daggers and slate pendants in graves must have lived on into the first period of the Bronze Age. Indeed one slate pendant, Luhm 6161, appears as a miniature shaft-hole axe of Bronze Age I.

The three pendants with an established position in relation to the skeleton suggest that the pendants were worn on a string round the neck. Signs of wear around the edge of the hole can be detected in certain cases but are never prominent and it should be noted that no less than 16 of the 225 pendants have no hole at all. Perhaps they were worn encased in some kind of receptacle?

As mentioned, only 45 of the 225 Scanian pendants are decorated. Decoration seems to have been the exception rather than the rule in the rest of Scandinavia as well. According to Hasselmo slate pendants without decoration are to be found in the region of the Horgener culture in Switzerland and in that of the Seine-Oise-Marne culture in France. Forssander (1936, p 101) stresses what would seem to be the confirmed contemporaneity of the stone cist period with Aunjetitz, and Hasselmo points out that the decoration of the slate pendants can be derived from the decoration of the metal objects of the Aunjetitz culture - as can be the style of metal decoration of the whole of the first period of the Scandinavian Bronze Age.

It seems clear that the wedge-shaped slate objects, with or without a hole, began as a type on the continent in areas where metal was in general use, and that they first appeared in Scandinavia during the late Neolithic, when metal had long been known. Forssander (1936, p 135) actually draws parallels between some of the Scandinavian types and others from central and western Europe.

The Scandinavian slate pendants have been interpreted in

a variety of ways. Glob (1965) assumes the ornaments to represent the earth mother's neck-ring, and thus assumes the slate pendants to be stylized images of the goddess. Hasselmo, on the other hand, points to the shaft-hole axes of the late Neolithic and emphasizes that the pendants provided with holes are not to be termed "whetstones". But there does not seem to be any difference in surface treatment or degree of wear between the pendants with holes and those without. They all seem to have been put to the same use.

Why was this type of artefact alone made of slate during the late Neolithic of south Scandinavia? Does black slate have any particular properties which might explain the function of the pendants and their distribution in time and space? The answer is obvious: black slate is still used today as a touchstone for ascertaining the approximate content of gold and silver alloys. The test is made by drawing a line on the touchstone with the object to be assayed and again with a sample of the metal of known composition. The colour of the lines is characteristic for each alloy. They can be erased with aqua regia, the speed with which the colour then disappears providing a further indication of the content of precious metal in the sample.

It appeared probable to the author that gold was not so common during the late Neolithic as to make it necessary for everybody or at least for many people to carry and use a touchstone. On the other hand copper and bronze were valuable and not exactly rare. No doubt it was important to many people to be able to determine the tin content of a bronze object with ease. Tests of modern bronze alloys on slate pendants have shown that it is easy to distinguish alloys with a 2 per cent difference in tin content and that even alloys that differ by less than 1 per cent

can be separated without any great difficulty, by the colour of the lines they leave when rubbed against the pendants.

Since the people of the late Neolithic certainly did not have aqua regia or other acids at their disposal, the lines must have been removed mechanically after each test, hence the wear.

The technical reason for the shape originally acquired by the pendants may have been as follows: since the marks had to be ground away, the surface nearest the hole was left unused so as not to damage the cord. In time even e.g. a rectangular-cylindrical pendants would become wedge-shaped, and it seems that this shape eventually came to be regarded as normal and desirable. The decoration of the pendants used in southern Scandinavia clearly indicates that they were formed in the shape of a wedge.

An examination of pendants constituting isolated finds and now in the custody of the Historical Museum of the University of Lund revealed visible coloured shades and marks on some of them most apparent on Luhm 2467 and 6161. An analysis of the pendants performed with the PIXE method (part II) showed three different gold-silver alloys, two on Luhm 2467 and one on Luhm 6161. The copper content was less than 1 per cent in the three alloys. When finding traces of gold on prehistoric artefacts, it is tempting to assume that they must be due to the excavator's wedding ring or graduation ring. This possibility can, however, be eliminated immediately. Nowadays, and since a very long time, gold is alloyed not only to modify its colour but also to increase its hardness. For this latter purpose, copper is invariably used in wedding rings and graduation rings. On the other hand alluvial gold almost invariably contains some silver - sometimes quite an appreciable percentage.

Luhm 2467 belongs to Hasselmo's type VC1, which is unfortunately not known from closed finds, and which thus cannot be dated more accurately than "late Neolithic or beginning of Bronze Age", whereas Luhm 6161 is a miniature slate copy of the heavy bronze shaft-hole axes of the first period of the Scandinavian Bronze Age.

Of course, traces of gold on two pendants out of about 50 examined does not prove that the pendants were not also used for assaying bronze. It will, however, probably remain impossible to prove it. Even if the late owner had neglected to grind away the surface marks of copper or bronze on the last occasion when he used the touchstone, we could not expect to find any traces of these metals on its surface. Copper and tin are both rather reactive, and thus the extremely thin traces of these metals on the surface of the touchstone would have been converted into soluble compounds after a few years underground. Gold, on the other hand, is stable and will survive in the ground.

Touchstones for bronze and precious metal in general use in late Neolithic times ! The idea seem incredible, but if correct it opens up a completely new perspective on the late Neolithic, the more so since alluvial gold is now known to occur in South Scandinavia.

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PART II

ANALYSIS OF WEDGE-SHAPED PENDANTS BY PROTON-INDUCED X-RAY EMISSION
SPECTROSCOPY (PIXE)

M Ahlberg, R Akselsson and B Forkman

Introduction

As mentioned in part I many of the pendants in the custody of the Historical Museum of the University of Lund show coloured shades and marks, the composition of which it appeared urgent to determine. Thus it was searched for a sensitive and non-destructive method for the analysis of the traces.

Johansson et al (1, 2) have shown that by proton-induced X-ray emission spectroscopy it is possible to detect 10^{-12} g or even less of many elements simultaneously, if they are deposited on a small area of a thin carbon foil, and that this method is sensitive to all elements with atomic number $Z \geq 15$.

Proton-Induced X-ray Emission spectroscopy (PIXE)

When a sample is irradiated by charged particles, atoms in the sample are ionized and excited. At the deexcitation characteristic X-rays are emitted. The energies of the X-rays are characteristic for the elements and the intensities are measures of the amounts of corresponding elements. Thus PIXE is a multi-element method. Figure 1 shows the experimental arrangement.

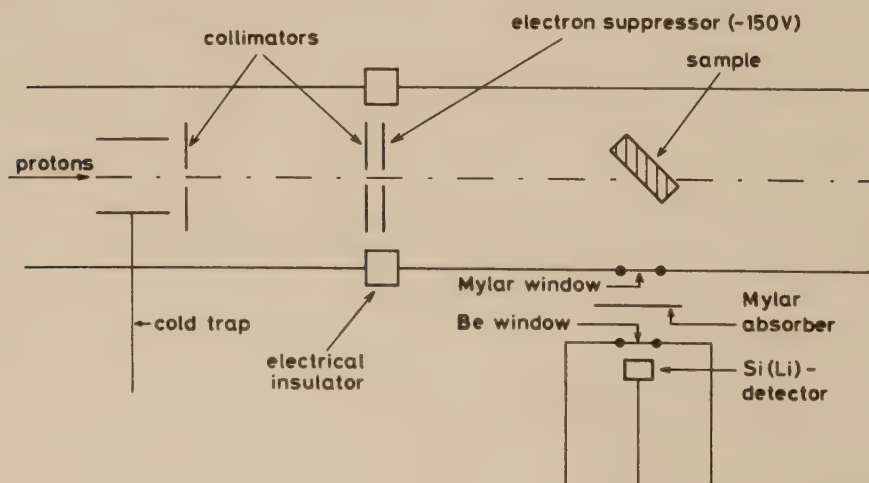


Figure 1 The experimental arrangement.

The pendants were irradiated with 2.5 MeV protons from a Van de Graaff accelerator and the X-rays were detected by a lithium-drifted silicon detector (area 11 mm², resolution 160 eV at 6.4 keV). The amplitude of the pulses from the detector is proportional to the X-ray energy and the pulses are analysed by a multichannel analyser. Figure 2 shows a typical pulse-height spectrum.

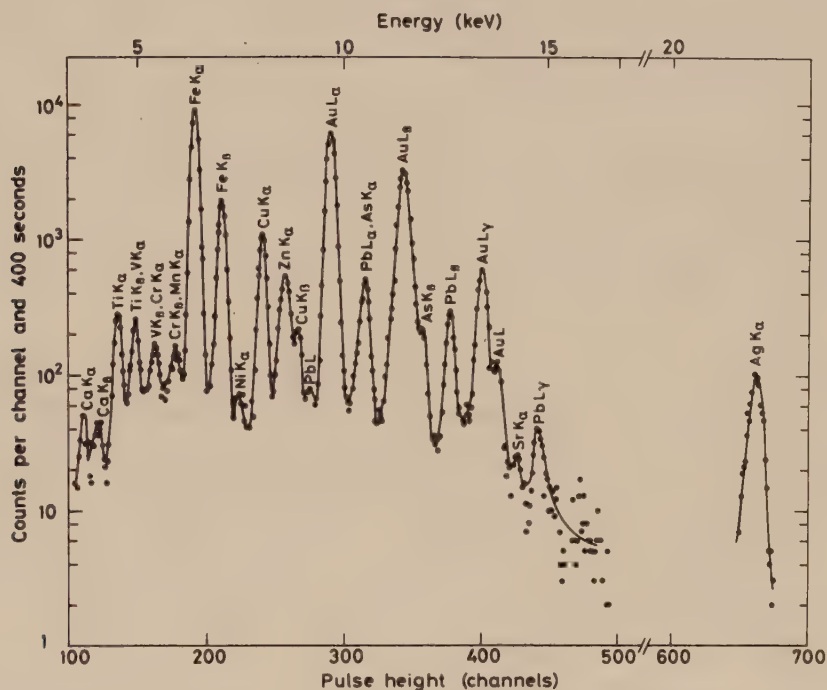


Figure 2 The pulse-height spectrum from the analysis of point 11 on luhm 2467. In this spectrum there are two characteristic peaks (K_{α} and K_{β}) from lighter elements and, in the main, three peaks (L_{α} , L_{β} and L_{γ}) from heavier elements.

In addition to the peaks corresponding to characteristic X-rays, there is a continuum according to scattered X-rays and according to bremsstrahlung from the deceleration of protons and secondary electrons. This is more apparent when irradiating thick samples and will reduce the sensitivity somewhat compared with that obtained by using thin carbon foils.

The number of pulses, Σ , in a peak from a certain element, Z , in a thin sample is given by

$$\Sigma = n N \sigma \omega k \Omega (4\pi)^{-1} \epsilon T \quad (1)$$

where

n = the total number of protons hitting the sample

N = the number of Z -atoms per cm^2

σ = the ionization cross section in cm^2

ω = the fluorescence yield

k = the relative transition probability

Ω = the solid angle subtended by the detector

ϵ = the efficiency of the detector

T = the transmission of the X-rays from the sample to the detector

This expression is valid if the X-rays come from the surface of the sample ($\leq 1 \text{ mg/cm}^2$). If, however, the X-rays come from atoms distributed in the sample, X-ray absorption and the decrease in X-ray production cross section due to the slowing down of the protons in the sample have to be taken into account, and the following expression is valid (3).

$$\Sigma = n C \omega k \Omega (4\pi)^{-1} \epsilon T_1 \int_{E_0}^{E_1} \sigma(E) T_2(\xi) (-dE/dx)^{-1} dE \quad (2)$$

where

C = the concentration of Z -atoms per cm^3

T_1 = the transmission of X-rays from the front face of the sample to the detector

T_2 = the transmission within the sample = $\exp(-\mu_m \xi)$

μ_m = the mass absorption coefficient in cm^2/g

ξ = the distance the X-rays have to pass in the sample in g/cm^2

$-dE/dx$ = the stopping power of the sample in MeV/cm

E_0 = the initial proton energy in MeV

E_1 = the final proton energy in MeV (0 if the protons stop in the sample)

Due to differences in σ and μ_m the thickness of the analysed layer is dependent on which element being investigated. Range estimations show that the thickness giving rise to 75 % of the pulses in a peak is about 15 μm for Ti and about 18 μm for Fe and heavier elements in slate, at an initial proton energy of 2.5 MeV. However, in the present investigation the gold-silver layer is thin enough for Equation 1 to be valid, as far as the determination of the composition of the layer is concerned.

Experiments and Results

In the study here presented seven pendants with different coloured shades and marks were investigated. Two of these showed apparent yellow marks which were suspected to be gold marks.

The pendants were mounted at 45° with respect to the beam direction and irradiated with a proton current of 25 nA in 400 seconds. The sample to detector distance was 51 mm and a 920 μm thick Mylar absorber (a plastics material) was inserted between the sample and the detector in order to reduce the low-energy part of the bremsstrahlung. In a first analysis the pendants were irradiated with an elliptical beam area of 27 mm^2 . In this examination traces of gold and silver were found on those two pendants which showed apparent yellow marks, luhm 2467 and luhm 6161. The other five pendants which had shades of different colours did not give any significant results of gold. The beam area was decreased to 2.2 mm^2 and the two pendants with gold and silver traces were investigated more carefully. Luhm 2467 was broken and the surface of fracture, which had not been directly exposed to the soil, was polished with carborundum to get a flat surface, washed with alcohol, and then analysed too.

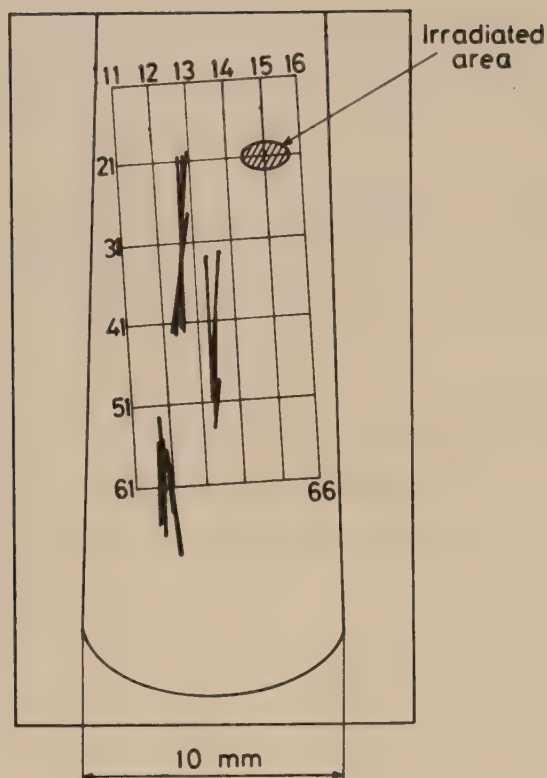


Figure 3

Luhm 6161 with the coloured traces and the analysed points.

Figures 3 and 5 show luhm 6161 and luhm 2467, respectively, and the analysed points and Figures 4 and 6 show the peak-height from some interesting elements in luhm 6161 and luhm 2467, respectively. (For silver the total number of pulses in the K_{α} -peak is plotted).

Point	23	33	42	43	44	53	54	61	62
Au (ng)	30	180	48	170	44	68	41	22	16
Ag (ng)	6.4	30	9.0	26	6.2	12	7.0	3.5	1.8
Au/(Au+Ag) (%)	82	86	84	87	88	85	85	86	90

Table 1 The detected amounts of gold and silver in ng and the percentage of pure gold for some points on luhm 6161.

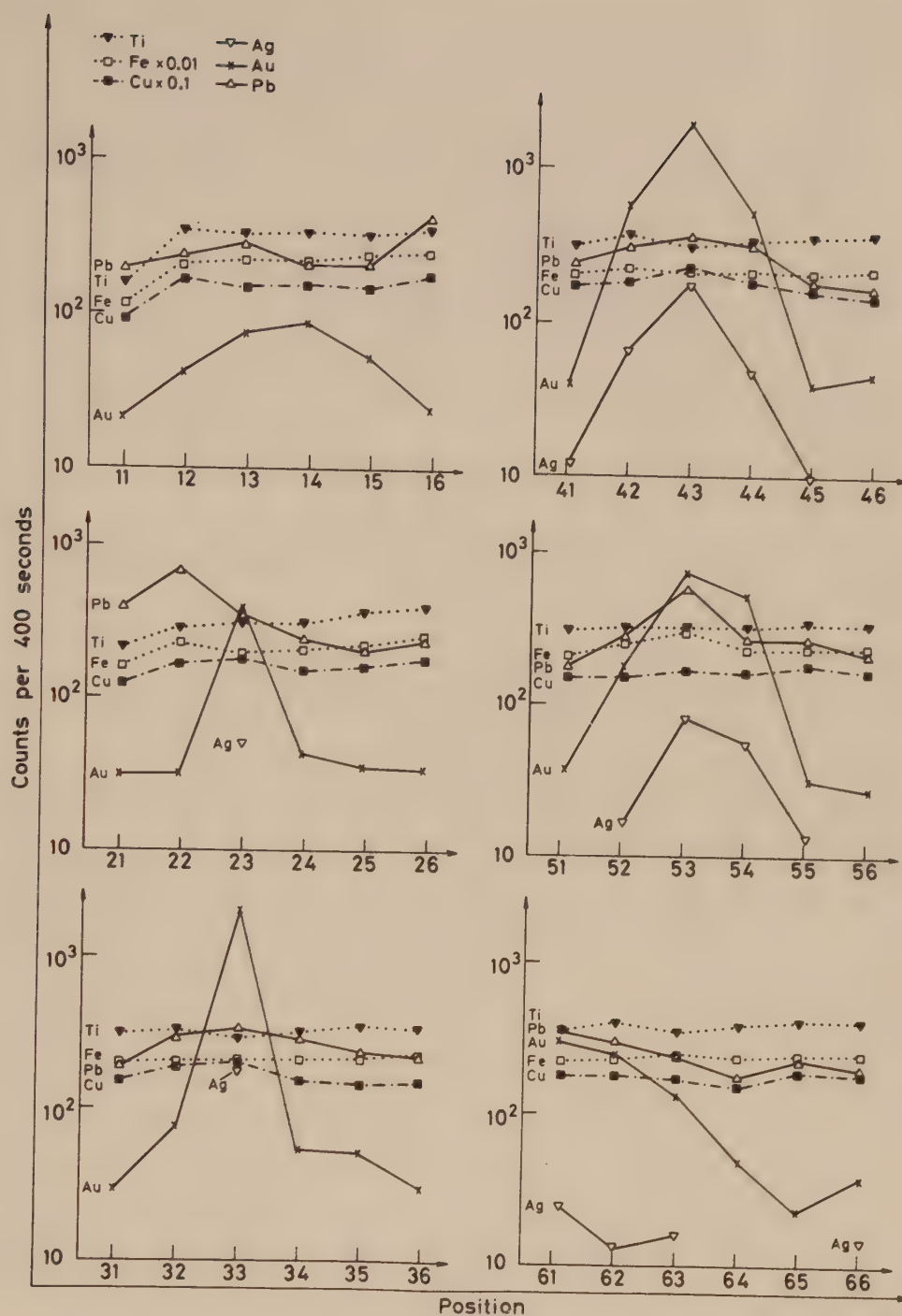


Figure 4 The peak-height for the elements Ti, Fe, Cu, Ag, Au and Pb from the analysed points in luhm 6161.

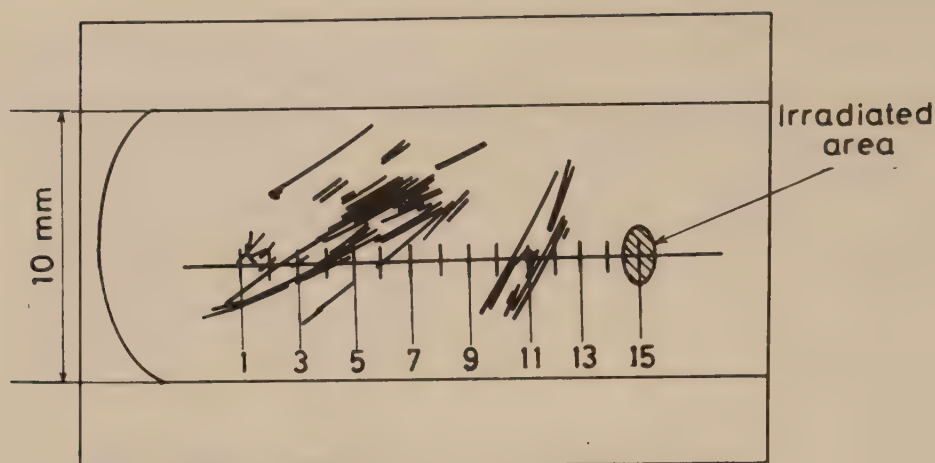


Figure 5 Luhm 2467 with the coloured traces and the analysed points.

Point	1	2	3	4	5	6	10	11	12
Au (ng)	350	540	470	390	310	170	110	520	130
Ag (ng)	7.2	7.4	9.0	6.0	7.9	5.6	28	140	42
Au/(Au+Ag) (%)	98	99	98	98	98	97	80	79	76

Table 2 The detected amounts of gold and silver in ng and the percentage of pure gold for some points on luhm 2467.

In both pendants there is to some extent a covariation between gold and silver. The other elements are rather constant (notice however lead) and show no covariation with gold and there is neither gold nor silver in the surface of fracture and therefore the coloured traces are supposed to consist of a pure gold-silver alloy. Tables 1 and 2 show the detected amounts of gold and silver as calculated by Equation 1, the calculated gold-silver layer is always less than $25 \mu\text{g}/\text{cm}^2$ and therefore this expression is valid.

In the points 7, 8, 9, 13, 14 and 15 in luhm 2467 there seems to be a background level of about 5 ng silver. This background has not been subtracted in the calculations of the percentage of gold in the alloys given in Tables 1 and 2 which may result in percentage values 1 - 2 % too low for the points 1 to 6 in luhm 2467.

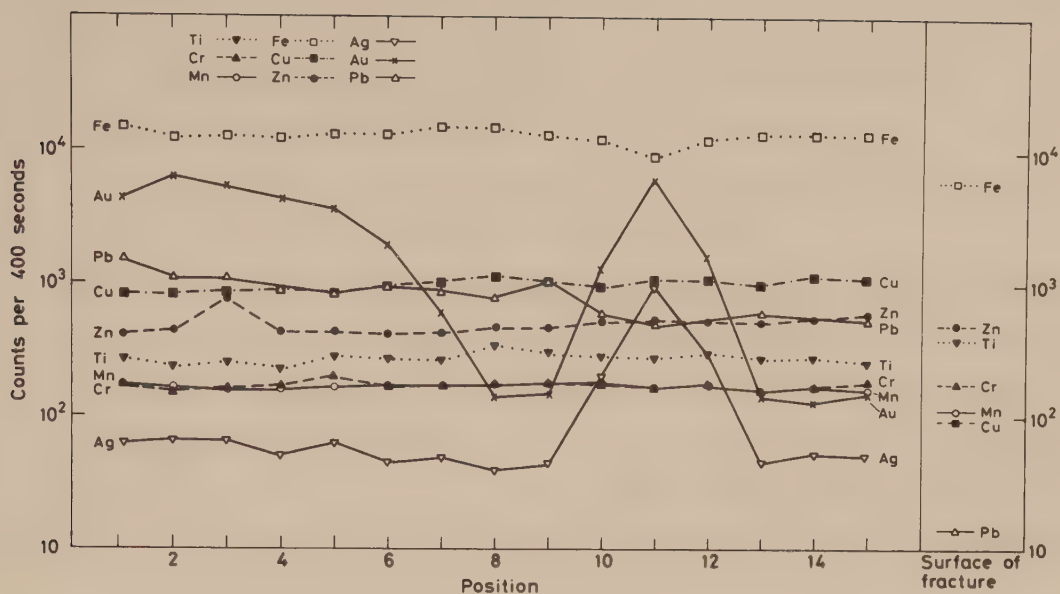


Figure 6 The peak-height for the elements Ti, Cr, Mn, Fe, Cu, Zn, Ag, Au and Pb from the analysed points of luhm 2467, including the surface of fracture.

From the values in Tables 1 and 2 three different gold-silver alloys can be derived : 85.9 ± 0.8 , 98.0 ± 0.3 and 78.3 ± 1.2 %. The copper content in the three alloys is less than 1 %.

Due to errors in σ_{wk} , Ω , ϵ and T there is an uncertainty of about 10 % in the absolute values. Also there is an uncertainty in the determination of the number of pulses, Σ , in a peak which for gold and the ratio gives rise to an error of less than 3 % for the points in the Tables.

In Figure 6 also the values for the surface of fracture are shown. Evidently there has been an exchange between the slate and the soil e.g. there is a much higher concentration of copper at the surface than in the slate.

Element	Ti	V	Cr	Mn	Fe	Ni	Cu	Zn	As	Rb	Sr	Pb
Concentration (ppm)	1000	170	45	4.3	1600	4.4	22	120	2.2	27	15	7.5

Table 3 The concentration of elements found in luhm 2467.

In Table 3 the concentration of elements found in the surface of fracture, calculated by Equation 2, is shown. In this calculations the values of stopping power and the mass absorption coefficients are computed from a standard shale (4). Together with the errors in the parameters in the equation this will cause a total error of about 30 % in the absolute concentrations.

The results are discussed in Part I.

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PROTON-INDUCED X-RAY ANALYSIS OF STEEL SURFACES FOR MICROPROBE PURPOSES

M. AHLBERG, R. AKSELSSON

Lund Institute of Technology, Lund, Sweden

D. BRUNE and J. LORENZEN

AB Atomenergi, Studsvik, Sweden

Received 31 May 1974

A study of the detection limits for the elements with $Z = 15-92$ in thick target steel surfaces using proton-induced X-ray technique has been performed.

Samples were irradiated with a broad proton beam of 2 mm diameter and the X-rays were detected by a Si(Li) detector. Detection limits at levels down to the order of 10 ppm were

achieved with simultaneous measurement of several elements.

Mylar and chromium absorbers were introduced in front of the semiconductor detector and irradiations at two different proton energies (1.0 and 2.5 MeV) were carried out in order to elucidate their effects on detection limits. The results are valid for microbeam analysis.

1. Introduction

Microprobe analysis is usually accomplished with the electron microprobe technique. However, by replacing the electrons with protons the bremsstrahlung induced in the sample matrix is considerably reduced and this implies lower detection limits for various elements.

In proton microprobe analysis¹⁻³) two techniques are used for spatial evaluation of an element in a surface, i.e. charged particle activation methods for the assay of light elements and proton-induced X-ray studies for the analysis of heavier elements.

With proton-induced X-ray technique and thin target arrangements extremely low detection limits can be obtained for several elements. This has been described previously⁴).

However, in studies comprising the determination of the distribution of a certain element in a metal surface using the proton microprobe and X-ray technique, detection limits are considerably reduced due to high background levels introduced in the thick targets exposed to the proton beam.

The present study concerns the evaluation of detection limits for elements with $Z = 15-92$ in thick steel samples using the proton-induced X-ray technique.

2. Theory

The number of pulses, Σ , in a certain peak from a certain element, Z , for a thin sample is given by

$$\Sigma = nN\sigma\omega k(\Omega/4\pi)\varepsilon T, \quad (1)$$

where n is the total number of protons hitting the sample with N Z -atoms per cm^2 , σ the cross-section for ionization of the specific shell⁵), ω the fluorescence yield⁶), k the relative transition probability^{7,8}), Ω the solid angle subtended by the detector, ε the efficiency of the detector and T the transmission of X-rays with a given energy through the absorbing material between the sample and the detector.

In the case of a thick sample (usually the case in material analysis) the number of pulses is given by:

$$\Sigma = nC\omega k(\Omega/4\pi)\varepsilon T_1 \int_{E_0}^{E_1} \sigma(E) T_2(\xi) (-dE/dx)^{-1} dE, \quad (2)$$

where C is the concentration (atoms/ cm^3), T_1 the transmission between the surface of the target and the detector, $T_2(\xi) = \exp(-\mu_m \xi)$ the transmission through the sample [μ_m is the mass absorption coefficient in cm^2/g ⁹) and ξ the distance the X-rays have to pass in the target in g/cm^2], $-dE/dx$ is the stopping power of the matrix¹⁰), E_0 the initial proton energy and E_1 the final proton energy in the target (here always 0).

The errors of the factors in eq. (1) have been fully discussed in ref. 5 and give a total error of 5% in N . The errors in μ_m and $-dE/dx$ in eq. (2) will increase the error in the determination of C to about 15%.

The energy spread of the protons is not taken into account in eq. (2), since for small depths the energy distribution is narrow and approximately symmetric, and in this narrow energy interval the cross-section is approximately a linear function of the energy, i.e. the increase in yield due to larger cross-section at higher

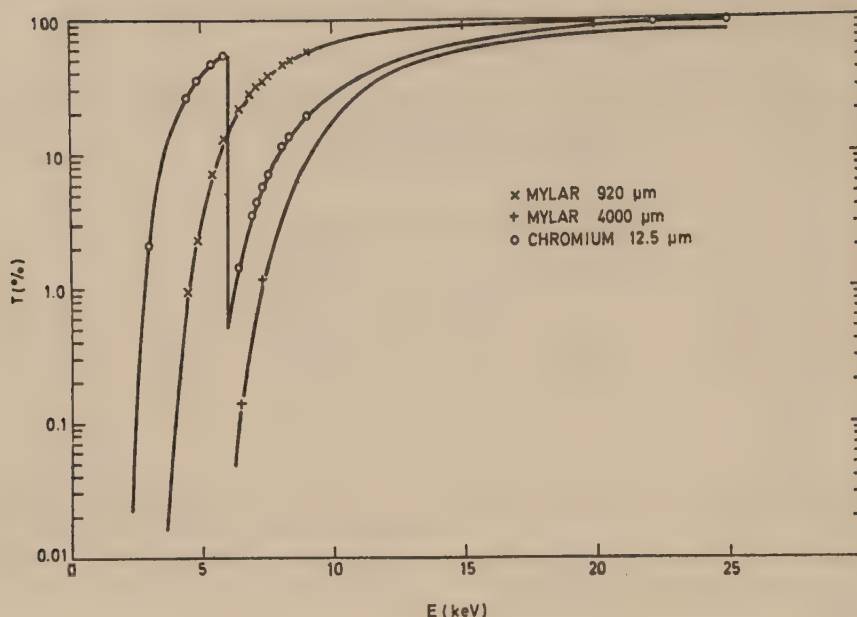


Fig. 1. Transmission of X-rays for 3 absorbers: Mylar (920 μm , 4000 μm) and Cr (12.5 μm).

proton energy is counteracted by the decrease in yield due to the lower cross-section at lower proton energy. In addition, at greater depth where the energy spread is considerable, both production cross-section and transmission of X-rays have decreased to a negligible part of the yield.

Secondary production of X-rays by higher energy X-rays, by secondary electrons and by bremsstrahlung are neglected. In analytical work this is not always permitted but for an estimation of detection limits these induced secondary X-rays are negligible.

Eq. (2) is independent of the diameter of the proton beam. That means that in a homogeneous material the lowest detectable concentration is the same for a microbeam as for a broad beam with the same proton current.

3. Different absorbers and proton energies

The counting rate in the detector is an essential factor for the detection limit. This rate is mainly caused by the characteristic X-rays from the major components in the matrix and by the bremsstrahlung spectrum induced by the secondary electrons. The bremsstrahlung spectrum is most predominant at low energies and decreases strongly with increasing X-ray energy. Furthermore it is practically negligible for energies higher than

$$E_e = \frac{4mM}{(m+M)^2} E_0, \quad (3)$$

where m and M are the electron and proton masses, respectively, and E_0 the initial proton energy. The bremsstrahlung yield as well as the X-ray production cross-section^{4,11}) in the MeV range increases with the proton energy. Two proton energies, i.e. 1.0 and 2.5 MeV were used giving E_e values equal to 2 and 5 keV, respectively.

The counting rate can be decreased by absorbers inserted between the target and the detector. The transmission increases with the X-ray energy except for the absorption edges. Fig. 1 shows the transmission as a function of the X-ray energy for the three absorbers used in this investigation. The theoretical curves have been normalized to some experimental points.

With a 920 μm Mylar absorber the contribution from the bremsstrahlung is decreased but characteristic X-rays with energies greater than 3.5 keV still have a transmission greater than 0.01%.

With a 4000 μm Mylar absorber the electron bremsstrahlung is completely absorbed. The characteristic X-rays from the major elements in steel (e.g. Cr, Fe, Ni) are also strongly absorbed. Thus this absorber is very useful for X-rays with energies higher than about 7 keV.

Mylar is composed of low Z materials which means that the absorption edges have low energies (<0.53 keV). Also the characteristic X-rays induced in the absorber have low energies and so they do not reach

the detector and cannot contribute to the counting rate. However, the K-absorption edge of chromium lies at 5.99 keV. With a $12.5\ \mu\text{m}$ chromium absorber only 1.5% of the Fe K_{α} -radiation is transmitted although the transmission at low energies is high (0.15% at 2.5 keV and 55% at 5.99 keV).

4. Experimental

4.1. SAMPLES

Two types of steel samples denoted A and B were analyzed. The composition of these samples is given in table 1.

4.2. MEASUREMENTS

The experimental arrangements are shown in fig. 2. The protons are accelerated by a 3 MV Van de Graaff accelerator and the beam is collimated to 2 mm diameter by two aluminium collimators. An Al-ring at

-150 V prevents backscattered electrons from disappearing out of the target chamber and permits a correct determination of the number of protons hitting the target by measurement of the charge. The target is mounted at 45° and the detector at 90° with respect

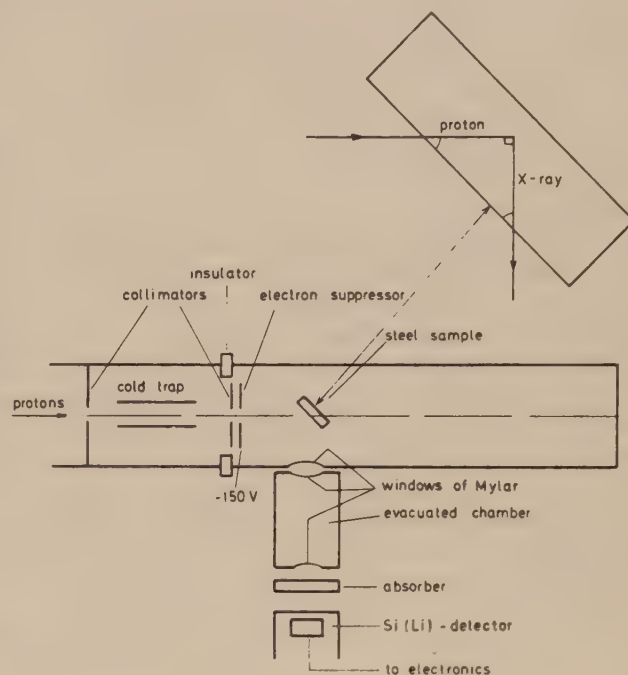


Fig. 2. Experimental arrangement.

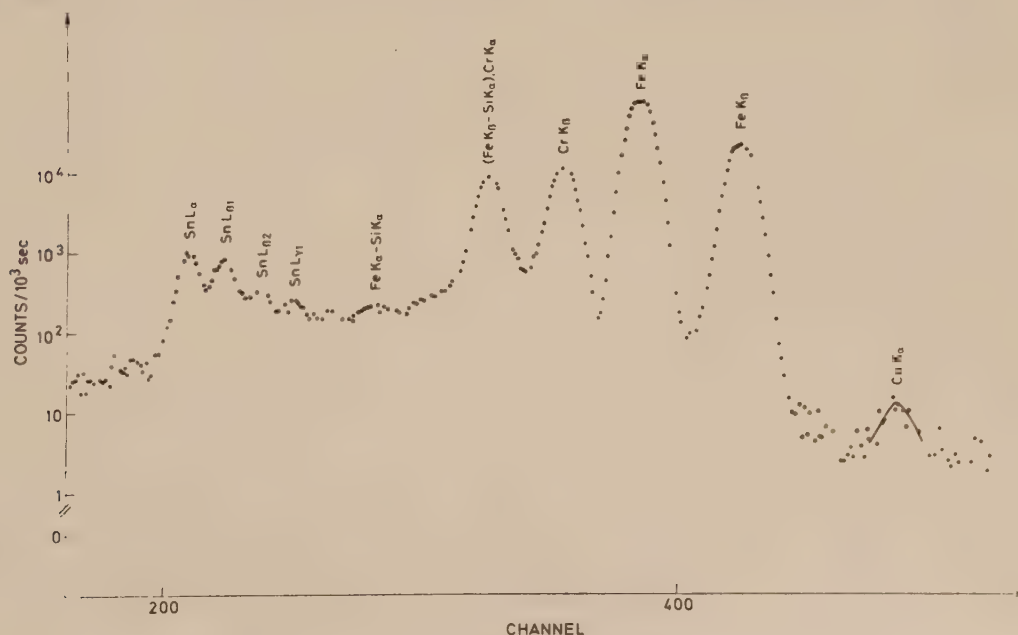


Fig. 3. X-ray spectrum of steel A at 1.0 MeV with Cr-absorber ($12.5\ \mu\text{m}$).

TABLE 1
Composition of steel samples.

Steel	Content (%)					
	C	Si	Mn	Cr	Ni	Mo
A	0.2	0.25	0.4-0.7			
B	0.04		1.5	17	12	2

TABLE 2
Experimental data.

Run No.	Steel	E_0 (MeV)	Absorber (μm)	Proton current (nA) corresponding to 1000 p/s
1, 2	A, B	2.5	4000 Mylar	1100, 575
3, 4	A, B	2.5	920 Mylar	7, 8
5, 6	A, B	1.0	920 Mylar	200, 240
7, 8	A, B	1.0	12.5 Cr	2200, 225

to the beam direction. The X-rays induced by the protons are detected by a Si(Li) detector (area=11.3 mm², depth=3 mm, fwhm = 160 eV at 6.4 keV). The pulses from the detector are analyzed by a multi-channel analyzer (1024 channels). An evacuated tube was inserted between the target chamber and the detector in order to reduce absorption effects in air. The sample to detector distance was 282 mm, of which 17 mm was air. With a pulse height discriminator at about 2.0 keV the pulse rate was adjusted to 1000 counts per second by changing the proton current. The running time was 1000 seconds. Figs. 3 and 4 show typical spectra from samples A and B.

In table 2 various experimental data are summarized.

5. Result and discussion

5.1. DETECTION LIMITS

The detection limit of an element is defined as the

concentration of that element which gives rise to a pulse number:

$$\Sigma = 3\sqrt{B}, \quad (4)$$

(or 20 for the case where $3\sqrt{B}$ is less than 20), where B is the number of background pulses in an energy interval equal to 2 fwhm around the peak energy. Figs. 5 and 6 show the detection limits obtained in this experiment.

5.2. DEPTH SENSITIVITY

In a thick sample only a surface layer is analyzed with the proton-induced X-ray method.

Due to differences in the energy dependence of the ionization cross-section for various elements and to the variation of the absorption coefficients with X-ray energy, the thickness of the layer which contributes to the yield will vary with X-ray energy, matrix and proton energy. The effect for steel A is shown in figs. 7a-c. A point on a particular curve tells how effective atoms at that depth are in producing a pulse in an X-ray peak compared to atoms of the same kind at the surface.

5.3. EFFECTS OF DIFFERENT ABSORBERS AND PROTON ENERGIES

Detection limits for the steel samples A and B are shown in figs. 5 and 6, respectively.

920 μm Mylar absorber: figs. 5a and 6a show that the 920 μm Mylar absorber is useful in a wide Z-region. Due to the energy dependence of the bremsstrahlung

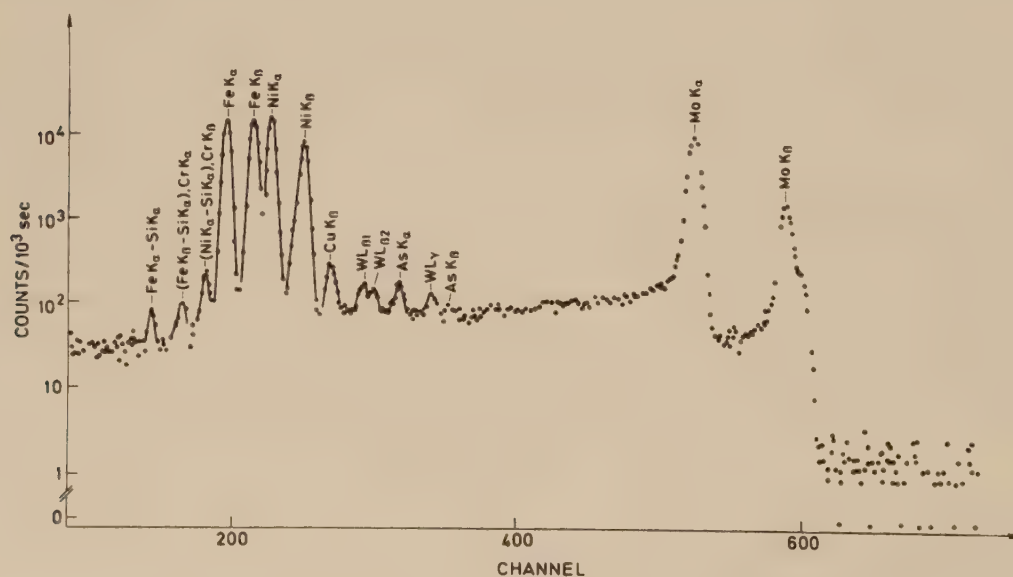


Fig. 4. X-ray spectrum of steel B at 2.5 MeV with Mylar absorber (4000 μm).

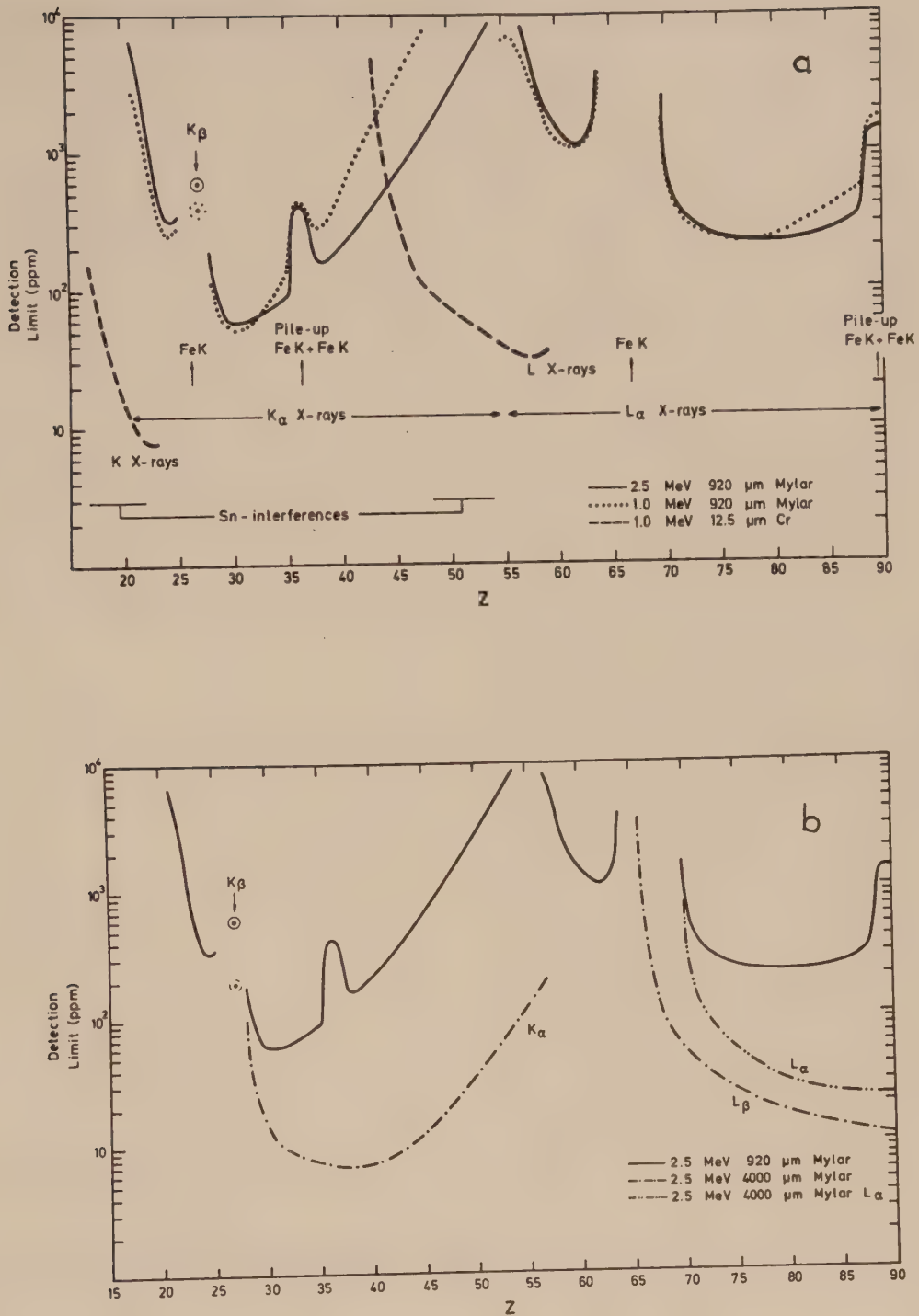


Fig. 5. Detection limits in steel A for different absorbers and proton energies. The criterium of 20 pulses in eq. (4) is used for the 920 μm Mylar absorber when $Z \geq 38$ for K X-rays and when $Z \geq 80$ for L X-rays. The discontinuities at $Z \approx 26$ and $Z \approx 65$ are due to interference with X-rays from the main component (iron). The anomaly at $Z \approx 52$ is due to interference with X-rays from the minor component Sn and the anomalies at $Z \approx 36$ and $Z \approx 89$ are due to pile-up and can be reduced. This is seen in the 4000 μm run as a result of the stronger absorption of the Fe K X-rays. (a) Absorber: 920 μm Mylar; 12.5 μm Cr. Energy: 1.0 MeV; 2.5 MeV. (b) Absorber: 920 μm Mylar; 4000 μm Mylar. Energy: 2.5 MeV.

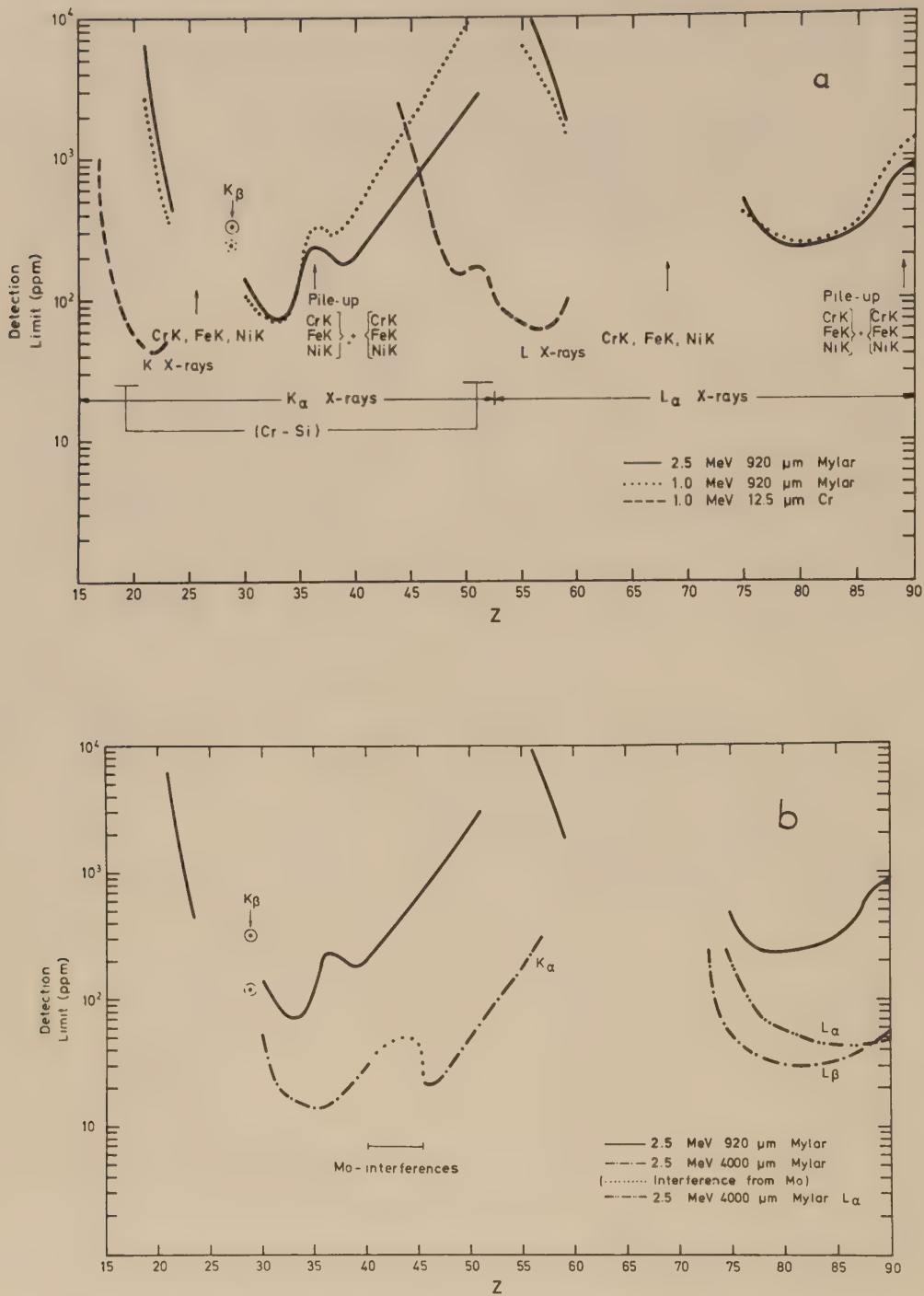


Fig. 6. Detection limits in steel B with different absorbers and proton energies. The criterium of 20 pulses in eq. (4) is used for the 920 μm Mylar absorber when $Z \geq 40$ for K X-rays. The discontinuities at $Z \approx 24-28$ and $Z \approx 60-72$ are due to interference with X-rays from the main components Cr, Fe and Ni. The anomalies at $Z \approx 36$ and $Z \approx 88-90$ are due to pile-up from the major components. They are reduced with the 4000 μm Mylar absorber. With this absorber there is an anomaly at $Z \approx 42-44$ due to characteristic X-rays from Mo and at $Z \approx 35-41$ due to imperfect charge collection of the Mo K X-rays. The anomaly at $Z \approx 51$ with the Cr absorber is due to Cr-Si escape peaks. (a) Absorber: 920 μm Mylar; 12.5 μm Cr. Energy: 1.0 MeV; 2.5 MeV. (b) Absorber: 920 μm Mylar; 4000 μm Mylar; Energy: 2.5 MeV.

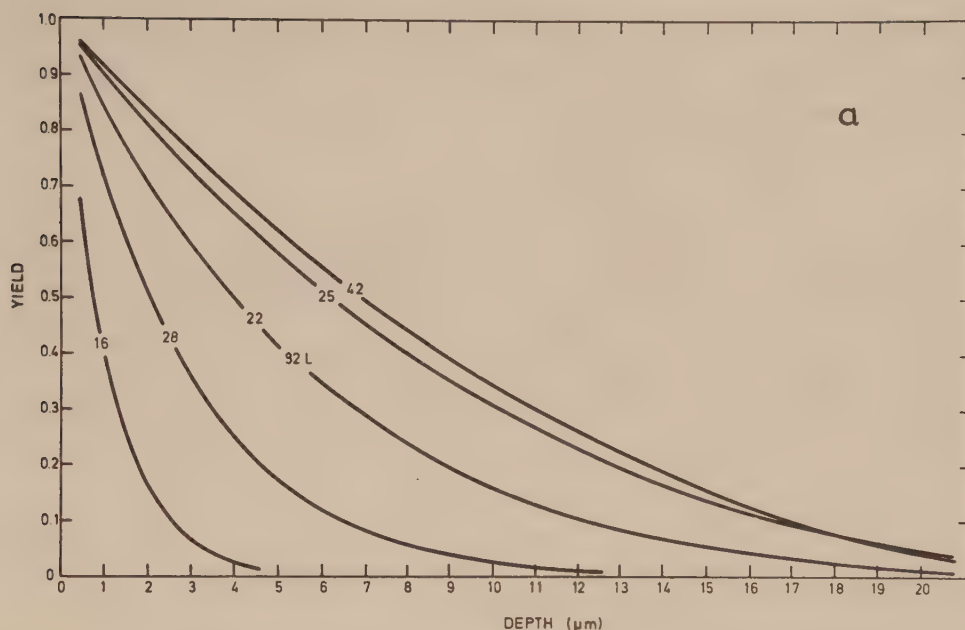


Fig. 7a. Effectiveness vs depth (according to eq. 2). Steel A at 2.5 MeV for various Z .

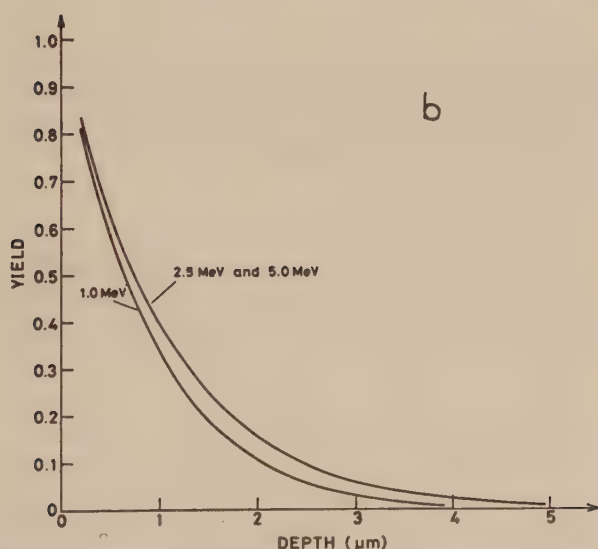


Fig. 7b. Effectiveness vs depth. Steel A for $Z=16$ at energies 1.0, 2.5 and 5.0 MeV.

the 1.0 MeV limits are lower than the 2.5 MeV limits for elements with low energy X-rays ($Z < 32$ for K X-rays and $Z < 77$ for L X-rays).

12.5 μm chromium absorber: figs. 5a and 6a show the feasibility of using a chromium absorber for elements emitting low energy X-rays. The chromium absorber is not so useful for steel B as it is for steel A since steel B itself contains chromium. The Cr K X-rays are of course on the low energy side of the Cr

K-edge which means a high transmittance why the contribution from Cr K X-rays to the counting rate becomes large.

The discontinuities at $Z \approx 26$ and $Z \approx 65$ are broad since characteristic X-ray lines with energies around those of Cr are not possible to detect because of the fluorescence production of Cr K X-rays in the absorber.

As seen in fig. 3 steel A contains a large amount of tin. This implies interference in the regions indicated in fig. 5a. With a lower amount of tin the limits with the Cr-absorber are decreased – by a factor of two for $Z = 19$ and for $49 \leq Z \leq 52$.

4000 μm Mylar absorber: figs. 5b and 6b show the limits of detection obtained with the 4000 μm Mylar absorber compared with those using the 920 μm Mylar absorber for 2.5 MeV protons. It is seen that the detection limit is increased in steel A by more than an order of magnitude for $Z > 32$ and for $Z > 66$, detecting K and L X-rays, respectively. With the thick absorber the detection limit is better for L_β than for L_α X-rays due to the better transmission and lower background for higher X-ray energies. The bump and discontinuity in the 4000 μm curve around $Z = 42$ in fig. 6b is due to interference from molybdenum in steel B. The degraded Molybdenum pulses increase the background significantly for elements with $35 \leq Z \leq 41$. This effect can be decreased by the use of a guard-ring detector¹²).

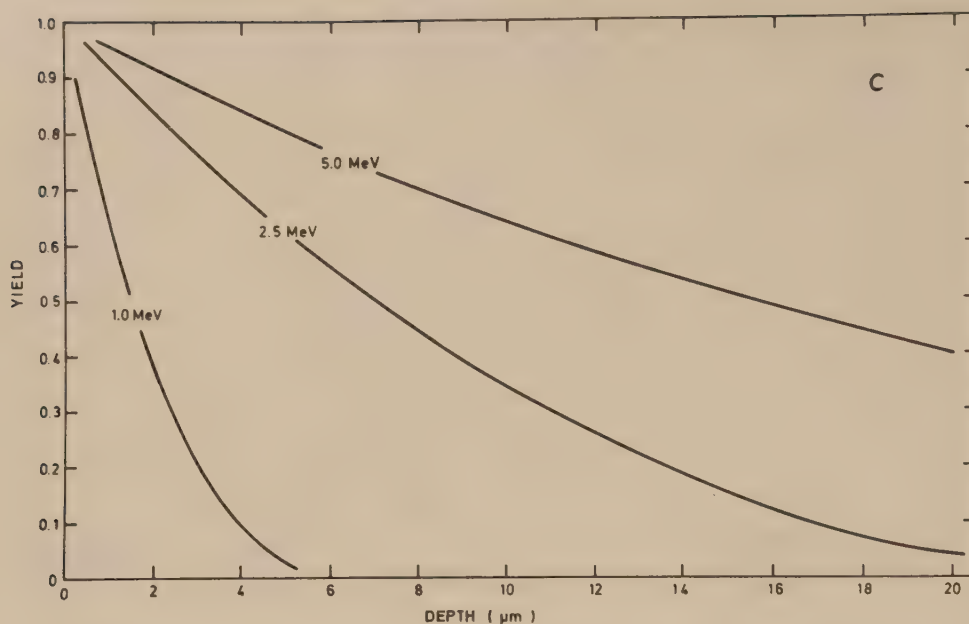


Fig. 7c. Effectiveness vs depth ξ . Steel A for $Z = 42$ at energies 1.0, 2.5 and 5.0 MeV.

TABLE 3
Minor and trace quantities found in the steel samples.

Steel	Contents of elements in minor and trace quantities (ppm)				
	Ni	Cu	As	Sn	W
A	100	190	40	720	—
B	—	2100	90	—	600

5.4. ELEMENTS ANALYZED IN MINOR AND TRACE QUANTITIES

Elements in minor and trace quantities (not reported by the manufacturer) found in the two steel samples analyzed are given in table 3.

5.5. THE DETECTION LIMIT PARAMETERS

With the criterium given in eq. (4) the detection limits decrease with increasing measurement time, t , as $t^{-1/2}$ and for elements for which the criterium corresponds to 20 pulses detection limits decrease as t^{-1} until 20 pulses are reached. A pulse rate of 1000 per second is relatively low and can often be increased: e.g. by increasing the proton current, by decreasing the target-detector distance or by increasing the

detector area. A change of the pulse rate changes the detection limit in the same manner as a change of the measurement time. At high pulse rates good pile-up rejection electronics is necessary.

5.6. ESCAPE PEAKS

The escape peaks due to silicon in the detector sometimes interfere with the peaks being measured. This effect can be eliminated by using a Ge detector since this device gives escape peaks at other energies.

5.7. MICROPROBE ANALYSIS AND DETECTION LIMITS

As pointed out above the detection limits are valid for any irradiated volume. Thus trace amounts of several elements can simultaneously be analyzed in volumes of the order of a few hundred cubic micrometers with a 10 μm diameter beam.

The results above are given under the assumption that the target is homogeneous regarding major elements. If volumes with other compositions are analyzed, as is the case for microprobe analysis, eq. (2) must be used with the correct superposition of stopping powers and the appropriate mass absorption coefficients. The detection limits are not altered very much. Interference with characteristic X-ray peaks, escape and pile-up peaks occurs, however, for other elements.

Financial support from the Swedish Board for Technical Development is hereby gratefully acknowledged.

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ELIMINATION OF CHARGING IN THE PROTON-INDUCED X-RAY
EMISSION ANALYSIS OF INSULATING SAMPLES.

M Ahlberg, G Johansson and K Malmqvist

Department of Nuclear Physics
Lund Institute of Technology, Lund, Sweden

Abstract

Anomalous background due to charging in the proton-induced X-ray analysis of insulators are eliminated by inserting a heated carbon filament or by raising the pressure in the target chamber.

Proton-Induced X-ray Emission (PIXE) has been shown to be a feasible analysing method especially for thin samples (1, 2). The high sensitivity obtained is partly due to the lower level of the continuous background radiation found in proton bombardment compared with that arising when electrons or photons are used for excitation (3, 4, 5). However, when the method is extended to the analysis of samples so thick that they stop the protons, the continuous background radiation is found to increase considerably when the samples are good insulators (1, 5, 6, 7, 8, 9). We have also seen such an effect in thick filters when the protons can pass through. The samples become charged by the proton beam, and, in the discharge, electrons are accelerated producing an intense background of bremsstrahlung which may reach out to several tens of keV. This bremsstrahlung reduces the sensitivity both due to the higher background level and to the fact that the proton current has to be decreased to adjust for the higher count rate in the detector.

This Letter describes two ways of neutralizing the charge build-up: by inserting a hot filament emitting electrons a short distance in front of the target, as proposed by Shabason et al (6), and by increasing the pressure in the target chamber. Both methods avoid the danger of contamination associated with the evaporation of conducting materials on to the sample (5, 7), or the mixing of target and conducting materials (10).

The filament in the "electron-gun" presented in Figure 1 is taken from a commercial carbon filament bulb. Tungsten filaments were tried but found to contaminate the sample severely when heated. The carbon filament is clamped to two carbon rods. These are fastened to a cylinder of plexiglass so that no heavy elements are heated when the glow current is turned on. A voltage of 7 V, which gave a glow current of 0.25 mA, was sufficient to neutralize the charge from a 50 nA beam of 2 MeV protons. To

prevent positive impurity ions from the carbon filament contaminating the samples, a perforated cap of aluminum kept at + 100 V was placed over the filament. The electron-gun was placed in front of the target in an insulated target chamber similar to that described in reference 11, the batteries being placed outside the chamber at atmospheric pressure. The electric connections were made as shown in Figure 1. The proton current could then be integrated for quantitative determinations without any interference.

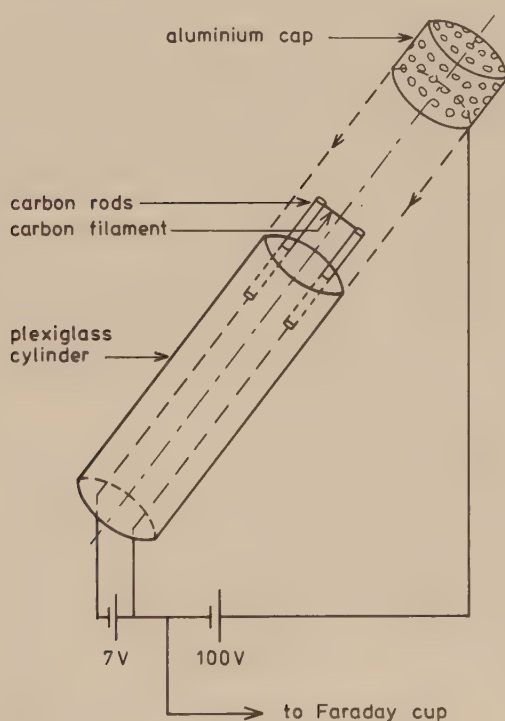


Figure 1

The electron-gun used to spray the sample with electrons.

Another simpler way of neutralizing the charge build-up on thick insulating samples was found to be by increasing the pressure in the target chamber. In the case of 2 MeV protons, the charging ceased when the pressure was raised to at least 1.5 Pa (≈ 10 microns). As the X-ray production cross section is a smooth function of proton energy, the slowing down of the protons due to the residual gas in the target chamber and by the very thin carbon layer which grows on the target at

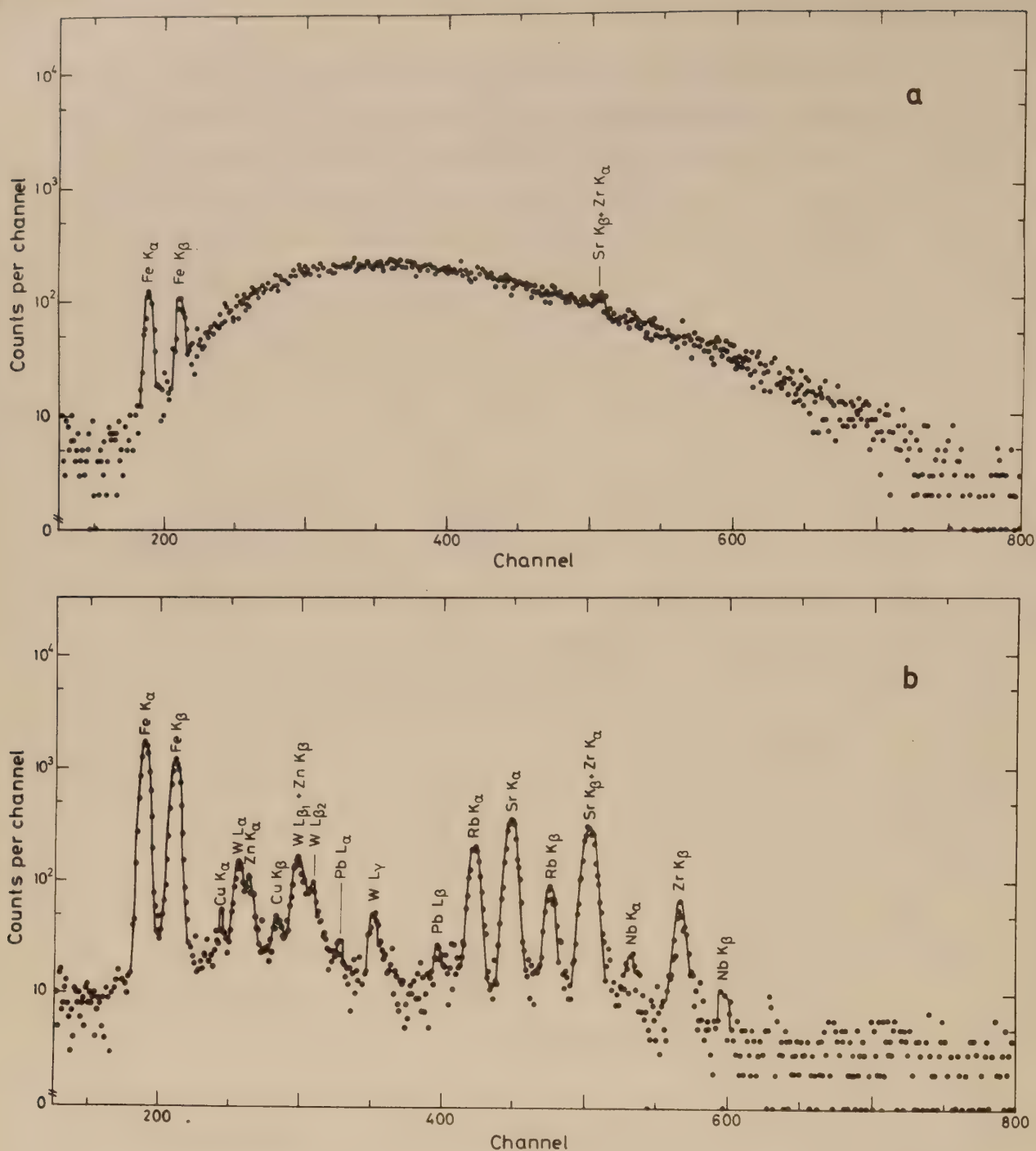


Figure 2 The spectra from a 3 minute analysis of a granite sample with 2 MeV protons, when the low energy X-rays are absorbed in a 280 μm thick aluminum absorber.

- When charging limited the proton beam to 3 nA.
- With a beam current of 50 nA, charging being eliminated by the use of a heated carbon filament in the target chamber.

low pressure, do not affect quantitative determinations. However, as the residual gas in the target chamber may be a source of impurities, the electron-gun method is to be preferred, when possible.

The drastic decrease in sensitivity due to charging is illustrated in the two spectra shown in Figure 2. Both show a 3 minute analysis of a powdered granite sample pressed to a pellet. When the spectrum in Figure 2a was recorded, charging limited the beam current to 3 nA which gave a count rate in the detector varying from 50 to 1 000 counts per second. When the electron-gun was turned on, the charging was eliminated and a beam current of 50 nA gave a steady count rate of 400 counts per second, resulting in the spectrum in Figure 2b. The spectrum obtained without the electron-gun but with the pressure in the target chamber raised to 3 Pa ($\approx$ 20 microns) was identical with that shown in Figure 2b.

The peaks correspond to 0.9 % Fe, 5 ppm Cu, 20 ppm Zn, 40 ppm Rb, 70 ppm Sr, 60 ppm Zr, 5 ppm Nb, 100 ppm W and 5 ppm Pb. When calculating the concentrations, the attenuation of the X-rays and the decrease in X-ray production cross section due to the slowing down of the protons in the sample have been taken into account (11).

We have benefitted from discussions with Drs R Akselsson and T B Johansson. We are grateful to Mr K Sjöberg for building the electron-gun, to Dr Z Solyom, Department of Mineralogy and Petrology, University of Lund, for providing the granite sample and to the Van de Graaff laboratory at the Niels Bohr Institute, Copenhagen for the possibility of performing this experiment.

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PROTON-INDUCED X-RAY EMISSION IN THE TRACE ANALYSIS
OF HUMAN TOOTH ENAMEL AND DENTINE.

Mats Ahlberg¹ and Roland Akselsson^{1,2}

1. Department of Nuclear Physics
Lund Institute of Technology, Lund, Sweden
2. Department of Environmental Health
University of Lund, Lund, Sweden

Abstract

In this study the feasibility of applying proton-induced X-ray emission spectroscopy (PIXE) to the trace element analysis of human tooth enamel and dentine is investigated. Detection limits on the order of 1 - 10 ppm are obtained. Depth yields for different elements and proton energies are discussed. Enhancement calculations show that the effect for PIXE-analysis is less important than for X-ray induced X-ray analysis. However, for elements with an absorption edge just below an intense X-ray line from a major element the enhancement effect is significant, and, due to the 40 % calcium in the enamel matrix, it is estimated to be 15 % for potassium. The importance of a smooth sample surface is discussed.

Introduction

Trace elements are often of fundamental importance in biochemistry. In dental tissues their presence is one of the factors which determines the degree of susceptibility to dental caries (1). Furthermore, teeth have been suggested as dose monitors for the exposure of man to foreign elements (2) as there are indications that the accumulation of elements in the dentine is proportional to the concentration in blood from which foreign elements often segregate fast. Considering the number of trace elements present and the possible interactions between them, multi-elemental analytical methods applicable to the trace analysis of human tooth enamel and dentine are of primary importance for the study of the role of trace elements. For survey purposes, of course, methods that require no predetermination of elements to be analysed are of special interest. Nuclear techniques offer several analytical methods applicable to the trace analysis of dental tissues. Neutron activation analysis has been the most used (3, 4, 5, 6, 7).

In recent years the fluorine depth distribution in human tooth enamel has been studied by proton activation analysis using the resonant nuclear reaction $^{19}\text{F}(\text{p}, \alpha\gamma)^{16}\text{O}$ (8, 9, 10). By also studying the resonant reaction $^{15}\text{N}(\text{p}, \alpha\gamma)^{12}\text{C}$ it has been possible to study the fluorine and the nitrogen depth distributions in developing dental enamel simultaneously, which is important as nitrogen is a measure of the amount of protein (11).

Due to the Coulomb barrier charged particle activation analysis is only applicable to light elements when using protons of a few MeV. In 1970 Johansson et al (12) demonstrated the feasibility of Proton-Induced X-ray Emission spectroscopy (PIXE) for fast multi-element trace analysis. PIXE has mostly been developed for thin samples ($\leq 1 \text{ mg/cm}^2$) (13, 14, 15, 16) although in recent studies PIXE-analysis of the surface of thick steel specimens (17) and thick pellets of biological materials (16) are reported. In this study we show the feasibility of applying PIXE to the trace analysis of human tooth enamel and dentine.

Theoretical Background

The yield of characteristic X-rays

In PIXE-analysis the proton-induced X-rays are detected by a Si(Li)-detector, and pulse-height spectra with peaks corresponding to emitted characteristic X-rays are obtained. The position of a peak in the pulse-height spectrum gives after calibration the X-ray energy and hence the element. The number of pulses, Σ , in a certain peak from a certain element of atomic number Z is given by

$$\Sigma = n_p C \omega k \Omega (4\pi)^{-1} \epsilon T_1 \int_{E_0}^{E_1} \sigma(E) T_2(\xi) (-dE/dx)^{-1} dE \quad (1)$$

here n_p is the total number of protons hitting the sample, C is the concentration of Z -atoms per cm^3 , ω the fluorescence yield (18), k the relative transition probability (19, 20), Ω the solid angle subtended by the detector, ϵ the efficiency of the detector, T_1 the transmission of the X-rays through absorbing material between the surface of the sample and the detector, σ the cross section for the ionization of the specific shell in cm^2 (21), $T_2(\xi) = \exp(-\mu_m \xi)$ the transmission through the sample (μ_m is the mass absorption coefficient in cm^2/g (22) and ξ the distance the X-rays have to go in the sample in g/cm^2), $-dE/dx$ is the stopping power of the matrix (23), E_0 the initial proton energy and E_1 the final proton energy (here always 0). The variation of ϵ and T_1 with X-ray energy and of $\sigma_p = \sigma \omega k$ with absorption edge energy is shown in Figure 1.

Matrix composition

In trace analysis, the trace elements to be determined exist in a so-called "matrix", which consists of the major constituents of the sample. In calculations with Equation 1 the stopping power and the mass absorption coefficient of the sample matrix have to be determined. For a composite sample they are calculated as weighted averages of the values for all major constituents in the sample. Hence the major composition of the sample has to be known. According to Brudevold and Söremark (24) enamel consists of hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, one per cent organic material and four per cent

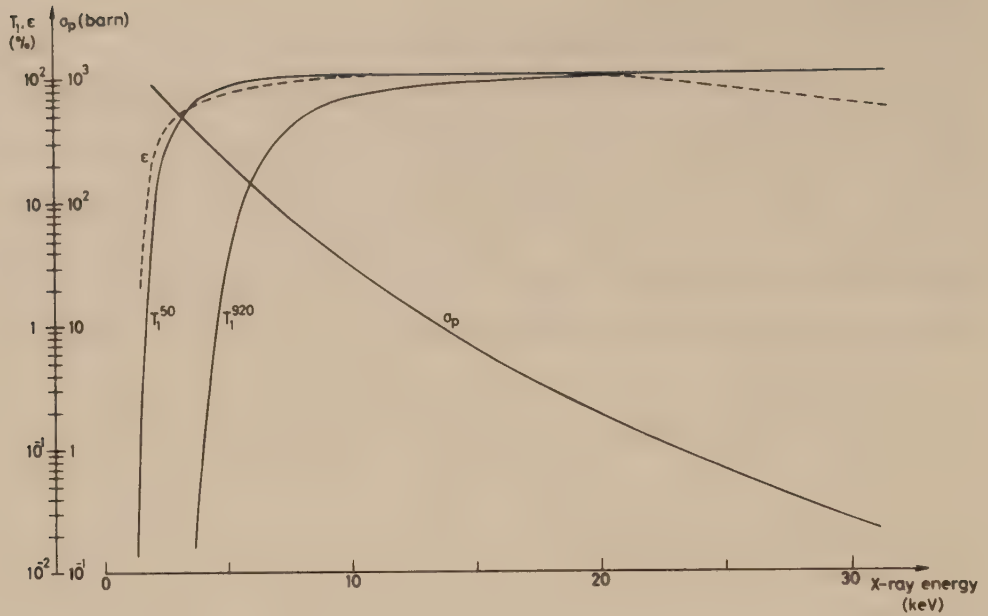


Figure 1 The transmission of the 50 μm and the 920 μm Mylar absorbers and the detector efficiency as a function of X-ray energy and the X-ray production cross section $\sigma_p = \sigma_{wk}$ as a function of K edge absorption energy for 2.5 MeV protons.

water. For the composition of dentine the concentrations of Na, P, and Ca are taken from Söremark and Samsahl (4) and of H, C, N, and O from Bowen (25) assuming the same relative abundance of these materials as in mammal bones. Table 1 shows the elemental composition of hydroxyapatite, the stopping power and mass absorption coefficient of which also are valid for fluorapatite, and the compositions we have used for enamel and dentine.

Enhancement effects

Enhancement effects in PIXE-analysis, i.e. secondary production of characteristic X-rays by proton-induced characteristic X-rays, secondary electrons and bremsstrahlung are neglected in Equation 1. The first effect gives under certain conditions significant contributions to the number of pulses in certain peaks. The cross section for proton-induced X-ray production is a decreasing function of the absorption edge energy (Figure 1) and the cross section for X-ray

Element	Hydroxyapatite	Enamel	Dentine
H %	0.2	0.6	2.6
C "	—	—	26.8
N "	—	—	4.3
O "	41.4	43.2	23.9
Na "	—	—	0.7
P "	18.5	17.8	13.5
Ca "	39.9	38.4	28.2

Table 1 Composition of the matrices used for the calculations of stopping power and mass absorption coefficients for Equation 1.

induced X-ray production is an increasing function of the same parameter as long as the energy of the primary X-rays is higher than the energy of the absorption edges. These facts imply that the enhancement effect is most important for elements with absorption edges just below an intense X-ray line and that the enhancement effect for PIXE-analysis is less than for X-ray induced X-ray analysis. This difference is larger the larger the difference is between the energy of the primary X-ray line and the energy of the absorption edge of the secondary element.

In a tooth matrix we only expect to get significant enhancement effects for elements with absorption edges below 3.69 keV, the Ca K_{α} X-ray energy. Table 2 shows a theoretical estimation of the enhancement effects in hydroxyapatite for 2 MeV protons.

Secondary X-ray	Primary X-ray	
	Ca K_{α}	Ca K_{β}
K K_{α}	15	1.0
Cl K_{α}	4.5	0.3
S K_{α}	2.5	0.2
P K_{α}	1.3	0.1

Table 2

The increase in X-ray yield in % due to enhancement from Ca K X-rays for the elements P, S, Cl, and K. Calculations performed for the hydroxyapatite matrix in Table 1 for 2 MeV protons and a geometry according to Figure 4.

The enhancement caused by an element 1 on an element 2 can be defined as the ratio between the total number of X-rays in a peak from element 2 and the number of X-rays in the peak induced by protons. If $I_p(1_2)$ is the intensity of primary induced 1_2 X-rays of element 2 and $I_s(1_1, 1_2)$ is the intensity of secondary 1_2 X-rays of element 2 induced by 1_1 X-rays from element 1, then the enhancement of 1_2 X-rays caused by 1_1 X-rays is

$$En(1_1, 1_2) = (I_p(1_2) + I_s(1_1, 1_2))/I_p(1_2)$$

En can be estimated by dividing the thick sample into thin slices according to Figure 2 and then calculating the number of primary and secondary X-rays, created in the different slices, which reach the detector. The middle of each slice is used as the starting point for all X-rays created in the slice. By making the slices thinner the approximation gets more accurate. Thus the enhancement is given by

$$En(1_1, 1_2) = (\sum_{nm} P_{s_n 1_1} Q_{s_n 1_1 s_m} R_{1_1 1_2} T_{s_m 1_2} + \sum_n P_{s_n 1_2} T_{s_n 1_2}) / \sum_n P_{s_n 1_2} T_{s_n 1_2}$$

$P_{s_n 1_i}$ is the primary 1_i X-rays of element i produced in slice s_n

$Q_{s_n 1_1 s_m}$ is the probability that an 1_1 X-ray produced in slice s_n is absorbed in slice s_m .

$R_{1_1 1_2}$ is the probability that an absorbed 1_1 X-ray produces an 1_2 X-ray

$T_{s_n 1_2}$ is the transmission for an 1_2 X-ray from slice s_n to the detector.

$P_{s_n 1_i} = n_p C_i \sigma_p(s_n) \sqrt{2} d_n$ according to Equation 1. $\sigma_p(s_n)$ is the production cross section for 1_i X-rays in slice s_n in cm^2 , and $\sqrt{2} d_n$ is the distance in cm the protons go in slice s_n according to Figure 2.

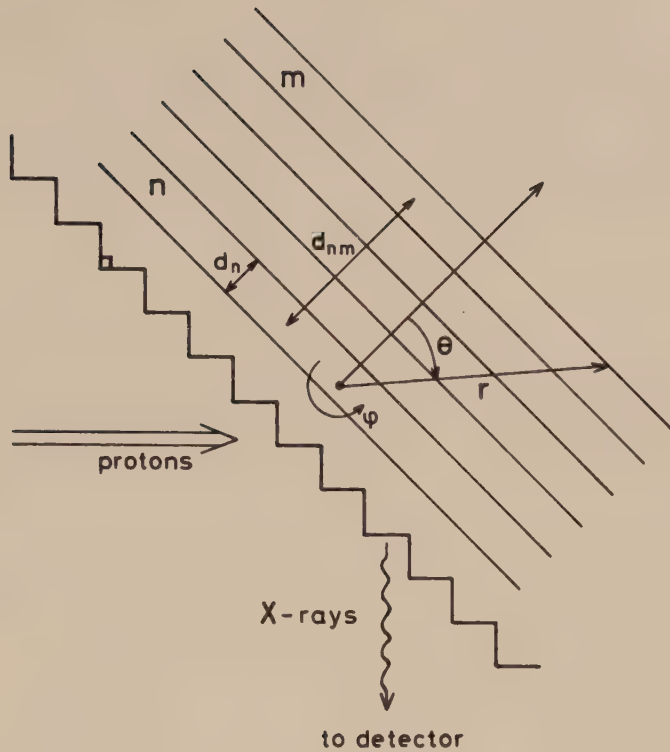


Figure 2

The slicing of the sample performed for enhancement calculations and the saw-tooth surface used for the estimation of roughness effects.

$$Q_{s_n l_1 s_m} = \int_{\theta=0}^{\pi/2} \int_{\phi=0}^{2\pi} \int_{(d_{nm}-d_m/2)/\cos\theta}^{(d_{nm}+d_m/2)/\cos\theta} \exp(-\mu_m(l_1)\rho r) (4\pi r^2)^{-1} \mu_m(l_1)\rho r^2 \sin\theta \, d\theta \, d\phi \, dr$$

d_{nm} is the distance from the middle of slice s_n to the middle of slice s_m and d_m is the thickness of slice s_m according to Figure 2. $\mu_m(l_1)$ is the mass absorption coefficient for l_1 X-rays in the matrix and ρ is the density of the matrix. When $n=m$ Q is obtained by integrating θ from 0 to π and r from 0 to $d_n/(2\cos\theta)$.

$R_{l_1 l_2} = \sigma_2(l_1) \omega k C_2 / (\mu_m(l_1)\rho)$, $\sigma_2(l_1)$ is the photo absorption cross section for l_1 X-rays in element 2 in cm^2 , ω and k the fluorescence yield and transition probability, respectively, for l_2 X-rays and C_2 the concentration of element 2 (atoms/cm^3).

$T_{s_n l_2} = \exp(-\mu_m(l_2) \xi(s_n))$, $\xi(s_n)$ is the distance the X-rays have to go in the sample from slice s_n in g/cm^2 .

Procedure

Samples

In order to check our procedures a flat standard of fluorapatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$, was analysed. The concentrations of the major elements in this matrix give about the same stopping power, mass absorption coefficients and yield of characteristic Ca and P X-rays as for an enamel matrix.

In this study results are given from analyses of one sound typical human tooth. The tooth was split up into two halves by first sawing two parallel furrows 0.5 mm deep and then pressing into these furrows by two sharp steel edges. In this way it was possible to get two uncontaminated surfaces. Four spots on one of the halves were analysed: the external enamel, the internal enamel, the dentine and the pulpal wall of the dentine. The positions of the analysed spots on the tooth are shown in Figure 3.

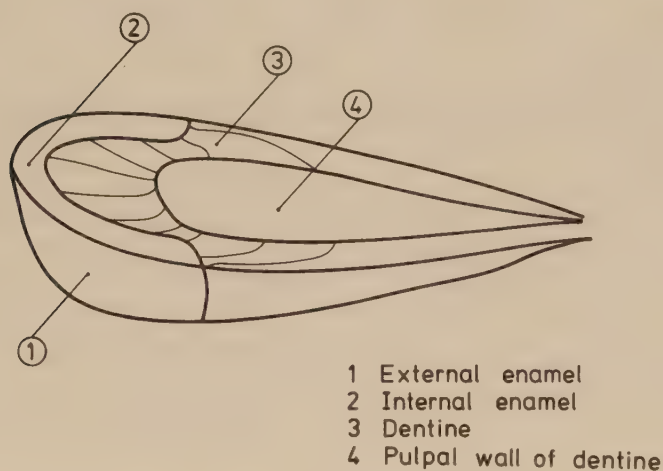


Figure 3

The position of the analysed spots on the tooth.

Absorbers

To reduce the count rate absorbers are inserted between the sample and the detector. The transmissions of the two absorbers used in this investigation are shown in Figure 1. The 50 μm thick Mylar absorber ($\text{C}_{10}\text{H}_8\text{O}_4$, $\rho = 1.38 \text{ g/cm}^3$) reduces very low energy X-rays and bremsstrahlung,

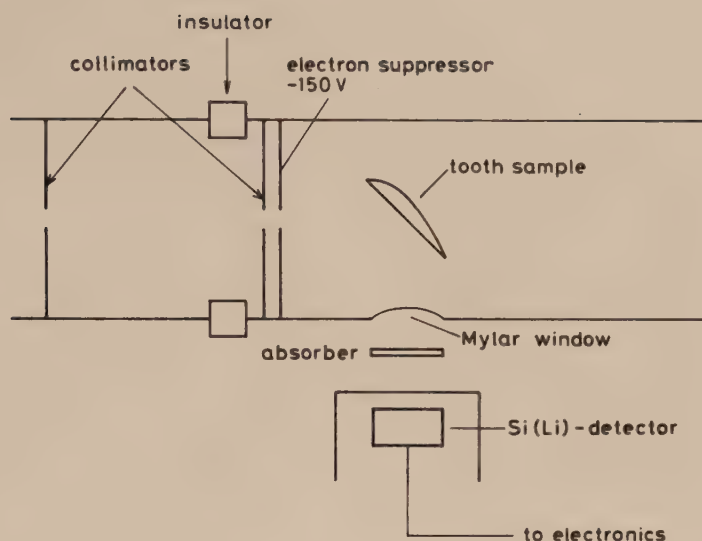


Figure 4 The experimental arrangement

still $P K_{\alpha}$ radiation has a 7 % transmission. With a $920 \mu\text{m}$ thick Mylar absorber the contribution to the count rate from X-rays lower than 3 keV is negligible (Figure 1). The transmission for $\text{Ca } K_{\alpha}$ radiation is 0.03 %. Thus this absorber is very useful for X-rays with higher energies and Ca can be detected for control and normalization purposes.

Measurements

The experimental arrangements are shown in Figure 4. Protons from the Lund 3 MV Van de Graaff accelerator were used. The proton energy was 2.5 MeV for the tooth irradiations and 2.0 MeV for the irradiation of the standard. The beam was focussed through two 1 mm diameter aluminum collimators. An aluminum ring at -150 V located between the last collimator and the sample was used to prevent secondary electrons from disappearing out of the insulated sample chamber. The integrated charge from the chamber was then used to determine the number of protons hitting the sample. The sample was placed with the surface to be irradiated at 45° with respect to the beam direction and the X-rays were detected by a Si(Li)-detector mounted at right angle to the beam direction according to Figure 4. The depth and the area of the detector

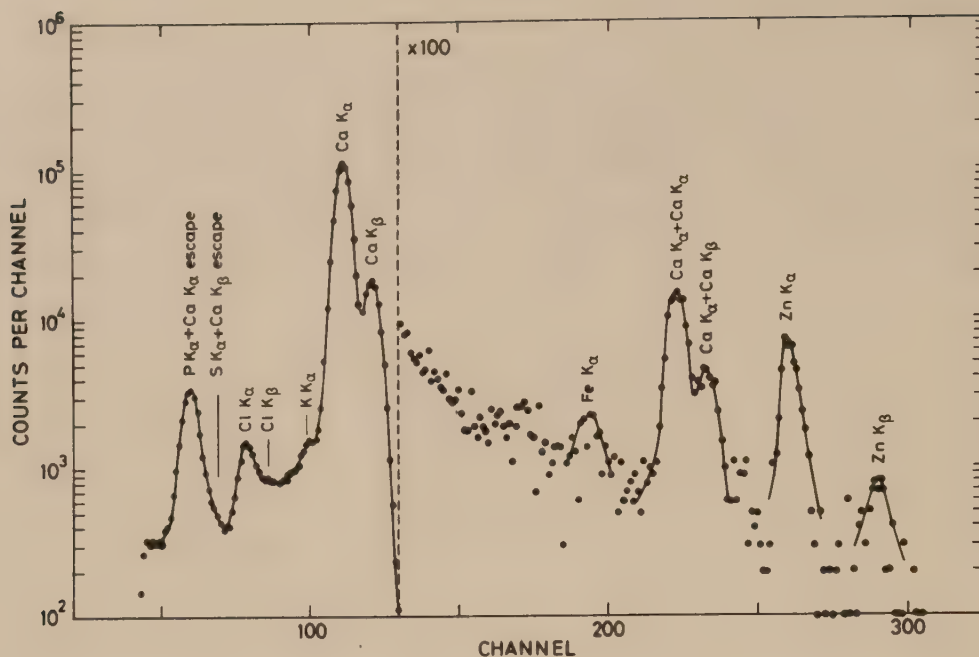


Figure 5 The pulse-height spectrum obtained from the analysis of the external enamel by using the 50 μm Mylar absorber.

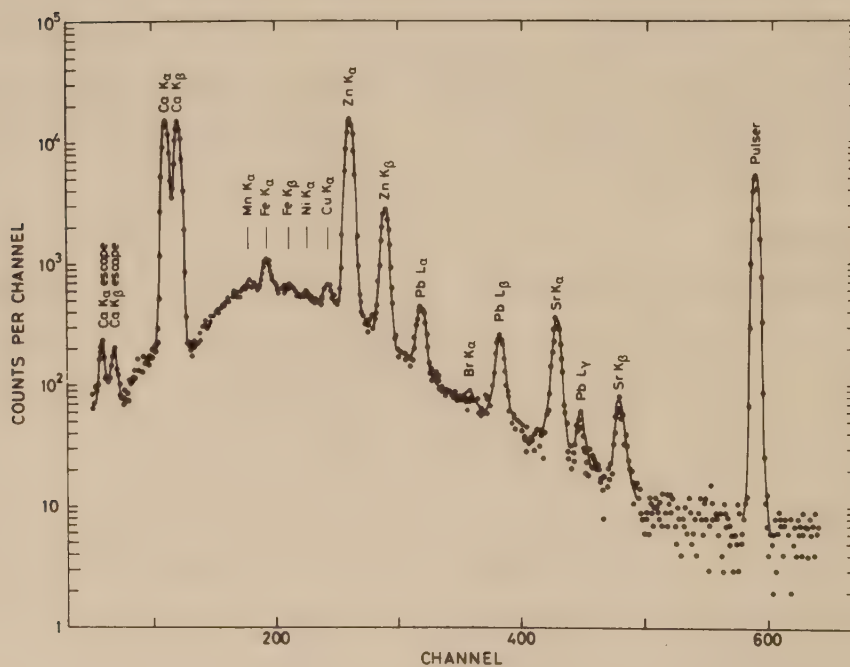


Figure 6 The pulse-height spectrum obtained from the analysis of the external enamel by using the 920 μm Mylar absorber.

were 3 mm and 11.3 mm², respectively. Figure 1 shows the efficiency of the detector the resolution of which was 160 eV FWHM (full width at half maximum peak-height) at 6.4 keV. The sample to detector distance was 51 mm including 10 mm of air and absorbers. The pulses from the detector were recorded by a multichannel analyser using 1024 channels. The counting rate was adjusted to 1000 counts per second by changing the proton current, which resulted in about 0.2 nA and 50 nA with the 50 μ m and 920 μ m Mylar absorbers, respectively. The analysing time was typically 600 seconds. Figures 5 and 6 show the spectra from the analysis of the external enamel with the two absorbers used.

The pulse-height spectra were analysed for X-ray peaks by the computer program SAMPO (26) which fits Gaussian peaks with exponential tails and a polynomial background to an interval with a maximum of six peaks at a time.

Charging

When a tooth is bombarded with protons in vacuum it becomes highly charged. In the discharge electrons are accelerated and produce bremsstrahlung of several keV which strongly reduces the sensitivity by raising the background level and the count rate. The charge build-up can be neutralized in two ways without contaminating the sample: by inserting a heated carbon filament emitting electrons a short distance in front of the tooth or as in this investigation by raising the pressure in the sample chamber to about 3 Pa ($\approx$ 20 microns). Both ways are fully described in another report (27).

Results and Discussion

Detection limits

Here the detection limit, DL, is defined as the concentration corresponding to

$$\Sigma = 3 \sqrt{B} \quad (2)$$

pulses in the peak. B is the number of pulses in the peak area (here taken as ± 1 FWHM centered around the peak position) which do not belong to the peak. Figure 7 shows the detection limits in internal enamel. Σ was greater than 20 for all elements shown in the figure. B differed less than 15 % between the different analysed spots. Dentine has a lower mass absorption coefficient, μ_m , and a higher stopping power ($-dE/dx$). Due to these effects DL for dentine is about 15 % less for low energy strongly absorbed characteristic X-rays (around 2 and 4 keV) and about 10 % higher for elements with more penetrating characteristic X-rays (around 30 keV).

The detection limits obtained with the 50 μm Mylar absorber are only given for elements with characteristic X-ray energies between 2.0 and 3.7 keV (P K_α and Ca K_α), because pile-up peaks occurred which interfered with characteristic X-rays of higher energies as seen in Figure 5. Furthermore the detection limits for elements emitting harder X-rays become lower by using the 920 μm Mylar absorber due to the better statistics obtained.

The discontinuities around $Z = 20$ for K X-rays and around $Z = 52$ for L X-rays are due to the interference with Ca K X-rays. Due to interference with X-rays from other elements always present in dental tissues some elements have to be determined by their less intense K_β peaks. This is the case for Ga and Zr the K_α peaks of which fall in the K_β peaks from Zn and Sr, respectively. Inherent with a silicon detector are the silicon escape peaks. In our case this effect is most apparent for Ca K X-rays as seen in Figure 6. These escape peaks fall in the P K_α and S K_α peaks thus increasing the uncertainty in the amount of phosphorus and the detection limit for sulphur.

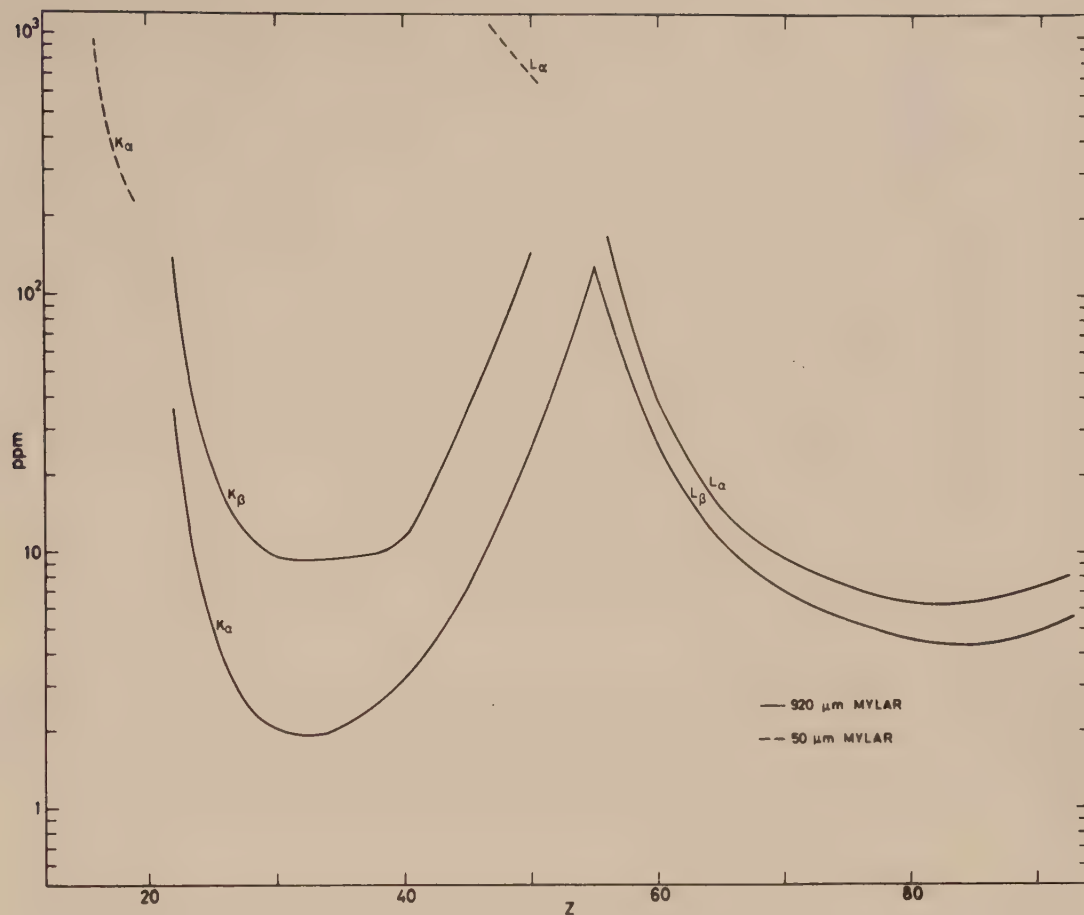


Figure 7 Detection limits in human tooth enamel with 2.5 MeV protons, 1000 counts per second, 600 seconds analysing time and two different Mylar absorbers with the arrangement shown in Figure 4.

These escape peaks can be avoided by using a Ge(Li)-detector which, however, introduces escape peaks at other energies.

For elements with low energy X-rays ($Z \leq 28$ for K X-rays and $Z \leq 70$ for L X-rays) the detection limits are increased with decreasing atomic number mainly due to the decrease in the detector efficiency and absorber transmission as shown in Figure 1. For elements with high energy X-rays ($Z \geq 40$ for K X-rays) the increase in the detection limits with increasing atomic number are due to the strong decrease in X-ray production cross section $\sigma_p = \sigma \omega k$ in Equation 1. In Figure 1 σ_p is shown as a function of the K absorption edge energy for 2.5 MeV protons.

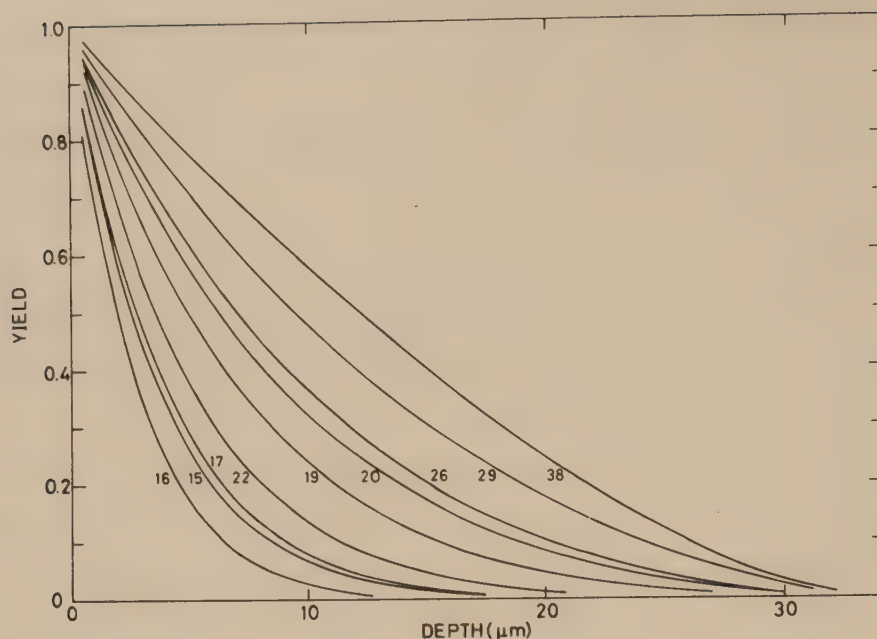


Figure 8a The depth yields for human tooth enamel, 2.5 MeV protons for selected values of Z from 15 to 38. Due to the high concentrations of P and Ca in the matrix, elements with characteristic X-ray energies just above the K edges of P and Ca such as S ($Z=16$) and Ti ($Z=22$) are strongly absorbed.

DL could be improved by improving the counting statistics by longer analysing times or by higher count rates. According to Equation 2

$$DL \sim t^{-1/2} \Omega^{-1/2} i^{-1/2}$$

here t is the analysing time, Ω the solid angle and i the proton current. An increase in Ω or i increases the count rate in the detector which may cause pile-up and bad resolution. Good electronics for pile-up rejection and pulse handling and an on-demand beam facility may now allow 5000 - 10 000 counts per second. An increase in the proton current density may cause problems as loss of volatile elements and destruction of the matrix. The solid angle could however easily be increased by using a larger detector and/or a shorter sample to detector distance. The detection limits obtained with the different absorbers can be achieved in a single run by using two detectors simultaneously. Good results can also be obtained with one detector by using a thick absorber with a hole in it as proposed by Cahill and Feeney (15).

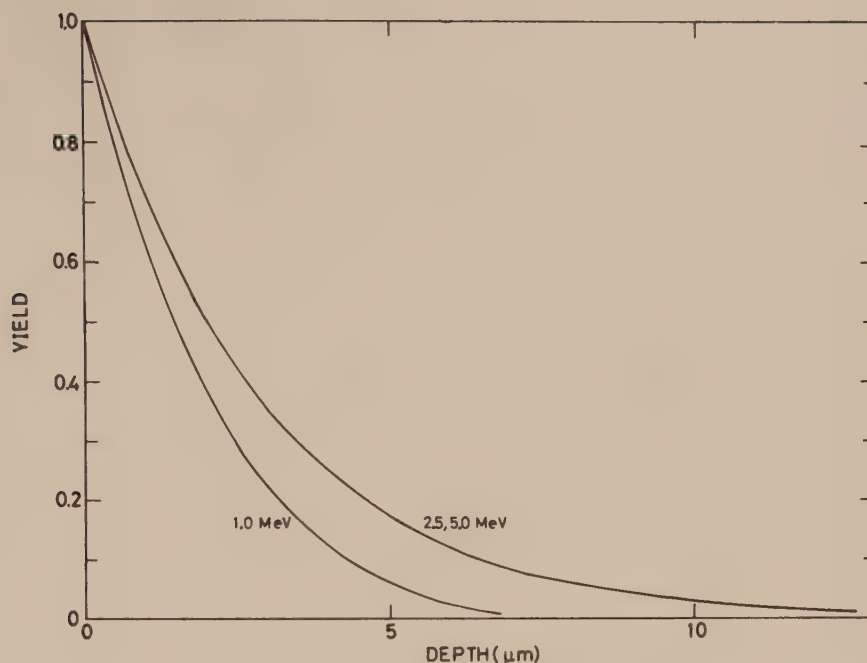


Figure 8b The depth yields for human tooth enamel, $Z = 16$ (S) for proton energies 1.0, 2.5 and 5.0 MeV. Due to the strong absorption of the soft S X-rays (2.3 keV) the deeper penetration of the 5.0 MeV protons does not affect the depth yield as compared with 2.5 MeV protons for this element.

A change in the proton energy changes the intensity of the continuum radiation and thus changes B. Also the cross section and the depth of the analysed layer are changed. Lower detection limits may be obtained for elements at the high energy ends of the K- and L-curves in Figure 7 by increasing the proton energy and for elements at the low energy ends by decreasing the proton energy (17).

Depth yield

Due to the slowing down of protons and the absorption of X-rays in the tooth only a surface layer is analysed, the thickness of which depends on proton energy, analysed element and the angles between the sample surface and the beam and between the sample surface and the sample to detector direction. Figures 8 a, b and c show the depth yield with atomic number and initial proton energy as parameters for the set-up used in this investigation. All curves are normalized to 1 at the surface. The calculations are performed for enamel assuming a density of 2.9 g/cm^3 (28).

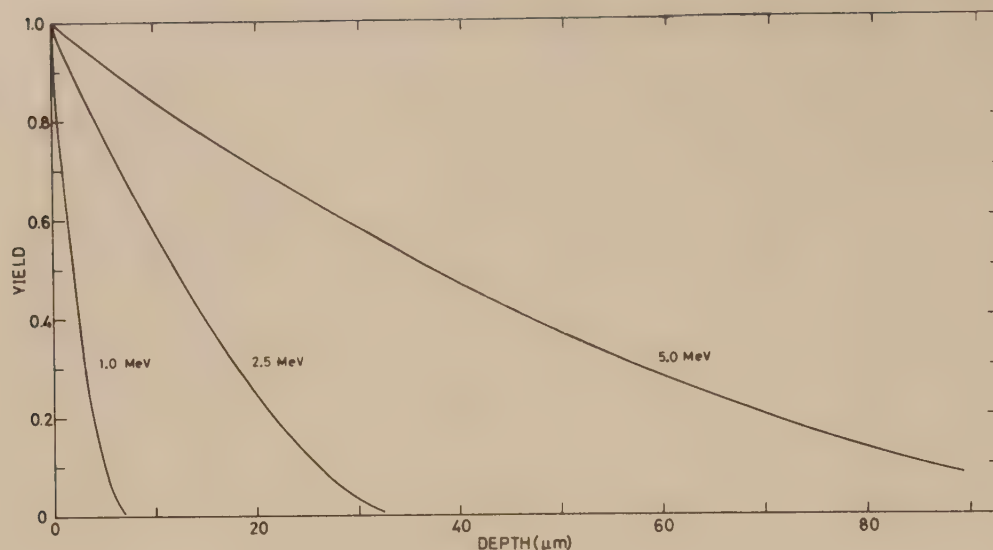


Figure 8c The depth yields for human tooth enamel, $Z = 38$ (Sr, K_{α} energy 14.2 keV) for proton energies 1.0, 2.5 and 5.0 MeV.

Surface roughness

In Equation 1 a flat sample surface oriented 45° both to the beam and to the sample-detector direction is assumed. In our attempts to achieve a smooth surface by sawing severe contamination mainly of Ti, Mn, Fe and Cu occurred. The splitting method described above eliminated the contamination, although a rougher surface was obtained. To estimate the surface roughness stereometer studies of electron microscopy pictures from split teeth were made.

The internal enamel showed a jagged surface with smooth hills and hollows. Height differences of about $30\ \mu\text{m}$, corresponding to $9\ \text{mg}/\text{cm}^2$, were estimated in a $0.5\ \text{mm}$ square. The dentine was penetrated by tubules about $1 - 2\ \mu\text{m}$ in diameter, which transversed the dentine from the pulp to the enamel in the plane of the surface of fracture. Along these tubules there were ridges and valleys. By orienting the tooth so that the tubules were parallel to the beam-detector plane, height differences of about $25\ \mu\text{m}$ could be decreased so that the

roughness as seen by the proton beam and the detector became about $10\text{ }\mu\text{m}$, in an area of 0.25 mm^2 . Assuming a density of 2.1 g/cm^3 for dentine (29) $10\text{ }\mu\text{m}$ corresponds to 2 mg/cm^2 .

When the surface roughness was approximated by a sawtooth function with a peak-angle of 90° , as shown in Figure 2, calculations showed that height differences of about 4 mg/cm^2 gave a decrease in the X-ray yield of less than 5% for X-rays above 2 keV, corresponding to all elements heavier than Si. A height difference of 9 mg/cm^2 decreases the X-ray yield as much as 30 % for X-rays between 2 and 4 keV. For X-rays of higher energies the decrease is less and above 8 keV it is below 10%.

The external enamel showed a smooth convex surface and the pulpal wall of dentine had a smooth concave surface in which the tubules occurred as holes. Due to these curved surfaces the angles in which the protons hit and the X-rays leave the surface deviate from 45° . For X-rays between 2 and 4 keV a change in angle of 5° changes the yield by about 15 %. Also this effect is a decreasing function of the X-ray energy and for X-rays above 8 keV the effect from a 5° change in angle is less than 5 %. The error due to the surface roughness could be decreased by mounting the detector as much as possible in the backward direction with respect to the incident proton beam.

The elements with characteristic X-rays of low energy (P, S, Cl, K, and Ca) which are most influenced by the surface roughness occur in rather large concentrations and therefore the surface could be polished without severe effects of the contamination on the determination of these elements.

Accuracy and Precision

The concentrations of the elements found in the four spots analysed on the tooth are given in Table 3. Our values for this single tooth are quite in agreement with the concentrations obtained with other methods on larger numbers (around 10 teeth). As concerns the results for the pulpal wall of dentine the high values of Ca and P indicate that the protons have penetrated the outermost layers and that most of the X-rays arise from the dentine. Depth gradients for some elements in the enamel are clearly seen.

The analysis of the fluorapatite standard gave Ca and P concentrations of $36 \pm 5 \%$ and $16 \pm 3 \%$, respectively, which is in good agreement with the values of $38.6 \pm 0.5 \%$ for Ca and $17.8 \pm 0.3 \%$ for P obtained by wet chemical methods. This agreement indicates that our procedure is reliable for a flat sample with a matrix similar to that of a tooth for elements with characteristic X-ray energies above 2 keV.

The uncertainties in the factors of Equation 1 give a total error of about 15 % in the determination of the concentrations. An additional error is obtained from the uncertainty in the matrix composition, in our case this is at most 5 %. The effect of the energy spread of the protons below the surface due to straggling is negligible (17).

Uncertainties due to sample roughness and target angle are the major uncertainties in the precision and one of the major uncertainties in the accuracy. The sample roughness always decreases the X-ray yield while an error in the target angle may decrease or increase the X-ray yield depending on whether the angle is greater or smaller than that assumed in the calculations. Thus a neutralizing effect is achieved for the curved surfaces of the external enamel and the pulpal wall of dentine. For elements in concentrations well above the detection limits and emitting X-rays with energies higher than 3.6 keV the precision and accuracy are typically 10 % and 20 %, respectively. For elements emitting softer X-rays the sample area to be irradiated has to be very carefully selected as regards surface smoothness.

Element		Enamel	Dentine	This work			
				External enamel	Internal enamel	Dentine	Pulpal wall of dentine
P	%	18.3 ± 2.2 a)	13.5 ± 2.8 b)	25	22	19	19
S	"	—	—	0.42	< 0.09	0.12	0.27
Cl	"	0.65 ± 0.30 a)	0.39 ± 0.11 b)	1.1	0.56	2.4	3.4
K	"	0.05 - 0.3 c)	—	0.22	0.07	0.06	0.16
Ca	"	37.4 ± 1.0 a)	28.2 ± 1.2 b)	35	30	27	25
Mn	ppm	0.54 ± 0.08 a)	0.19 ± 0.06 b)	10	< 5	< 5	< 5
Fe	"	338 ± 109 d)	110 ± 22 e)	48	9.0	5.5	14
Ni	"	—	—	4.5	< 2.5	< 2.5	4.0
Cu	"	0.26 ± 0.11 a)	0.21 ± 0.10 b)	18	< 2.2	< 2.2	13
Zn	"	276 ± 106 a)	199 ± 78 b)	1200	40	150	510
Br	"	4.6 ± 1.1 a)	4.0 ± 2.0 b)	2.5	< 2.2	6.0	7.9
Sr	"	94 ± 22 a)	70 ± 18 b)	120	170	200	190
Pb	"	—	—	140	50	8.9	29

a) Söremark and Samsahl (3)

b) Söremark and Samsahl (4)

c) Compiled from the literature (24)

by Brudevold and Söremark

d) Söremark and Lundberg (5)

e) Söremark and Lundberg (6)

Table 3 Concentrations of elements found in one tooth by PIXE-analysis.
For the elements calcium and heavier the error is typically 20 %
whereas it is greater for lighter elements due to surface roughness.

An opportunity of testing the accuracy and precision of PIXE on thick samples using Equation 1 was offered in an intercomparison study performed during spring 1974 (30). Twenty-two investigators collectively used six different techniques on three different kinds of samples. One of these was a dried solution deposit in a Millipore cellulose membrane filter which exposed a homogeneous sample of 6 mg/cm^2 thickness to the proton beam, when mounted at 45° with respect to the beam direction. The elements deposited comprised S, K, V, Cr, Mn, Fe, Zn, Cd and Au with

Element	PIXE Lund/ gravimetric value	PIXE Lund/average of central two-thirds of reported values
S	1.14	1.02
K	0.84	0.85
V	0.88	0.91
Cr	0.91	0.98
Mn	0.91	0.98
Fe	0.89	0.96
Zn	0.88	0.96
Cd	0.82	0.86
Au	0.77	0.74
Mean and Standard deviation	0.89 (0.10)	0.92 (0.09)

Table 4 Results from an intercomparison study. One standard deviation was calculated to be about 15 % for PIXE.

concentrations in the range 1 to 50 $\mu\text{g}/\text{cm}^2$. This sample decreased the energy of our proton beam from 2.5 MeV to 1.5 MeV which, for the most influenced line Cd K, means a reduction in the X-ray production cross section to one fifth of the initial value. The transmission of S K_{α} X-rays through 6 mg/cm^2 of the sample is about 17 %. Correction factors by means of Equation 1 to thin sample values were 3.3 for S, 2.0 for elements around Cr and 2.5 for Cd, all using K X-rays. In Table 4 the ratios firstly of our results and gravimetric values and secondly of our results and the means of the central two-thirds of the values reported in the intercomparison study are given. The means and standard deviations given in the table support the values of accuracy and precision estimated above for PIXE-analysis on thick tooth samples.

Conclusions

Proton-induced X-ray emission analysis is shown to be a method with good applicability for contamination free analysis of elements heavier than calcium in enamel and dentine. The analysis is rapid and gives low detection limits. The precision and accuracy is typically 10 % and 20 %, respectively.

By carefully polishing the surfaces it will also be possible to analyse elements giving softer X-rays with a good accuracy and precision.

At this laboratory the method is now used on a large scale for determination of heavy elements in children's teeth with special interest in lead.

Acknowledgements

We very much appreciate the encouraging discussions with Professor Maths Berlin, Dept. of Environmental Health, Professor Lars Hammarström Dept. of Oral Histopathology, and Professor Sven A E Johansson, Dept. of Nuclear Physics. Also we are grateful to Mr Knut Sjöberg in the machine shop, and to all members of the VdG staff and the PIXE group for valuable discussions and help. The 25 year old accelerator which gave us its last protons for this experiment should be remembered in honour.

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SIMPLE DEPTH PROFILE DETERMINATION BY PROTON-INDUCED
X-RAY EMISSION

M Ahlberg

Department of Nuclear Physics
Lund Institute of Technology, Lund, Sweden

Abstract

This letter examines the possibility of determining in proton-induced X-ray emission simultaneously both the surface concentration and the concentration within the sample of an element by measuring its β/α -ratio.

Since the introduction of Proton-Induced X-ray Emission (PIXE) in trace analysis by Johansson et al 1970 (1), PIXE has been developed into a reliable tool for the routine multi-element analysis of thin samples (2, 3). Recently, we have applied PIXE to the trace analysis of thick samples. Detection limits have been derived for thick specimens of steel (4) and for samples of human tooth enamel and dentine (5). In addition, an investigation of thin gold layers on samples dating from the Stone Age have been performed (6). In these analyses a simple method of determining whether an element is located on the surface of the sample or distributed within it was found to be of great importance.

Hitherto, two possibilities of obtaining depth distributions in PIXE-analysis have been suggested: the variation of the incident proton beam energy (7, 8), and the variation of target angle (θ in Figure 1) (9, 10, 11). Reuter and Smith (7) showed that the variation of proton energy allows only one to two unknowns to be determined in a depth distribution. Pabst (10) found strong oscillations due to experimental errors in the calculation of concentration profiles from data obtained by varying the target angle θ . However, he was able to overcome some of the difficulties by comparison of measured yields as a function of target angle with theoretical curves and by including θ -values close to 0 (11). The two methods described above need several careful measurements of the X-ray yield under different conditions.

However, due to the energy dependence of the mass absorption coefficient, the β/α -ratio is greater for an element when it is distributed within the sample than when it is located as a thin surface layer, since the energies of K_β and L_β X-rays are greater than those of corresponding K_α and L_α X-rays. Thus, it will be possible to determine the surface and sample matrix distribution of an element by measuring its β/α -ratio. Since both the α and β X-ray lines from an element are recorded simultaneously in an energy dispersive X-ray system, their relative intensity ratios,

i.e. the distribution between surface and sample matrix is obtained in a single run. Furthermore, the distribution of the element can be very accurately determined since the uncertainty in X-ray yield due to errors in the charge measurement and determination of the solid angle subtended by the detector, does not affect the distribution, but only the absolute concentrations. This is in contrast to those depth profile measurements which require more than one irradiation.

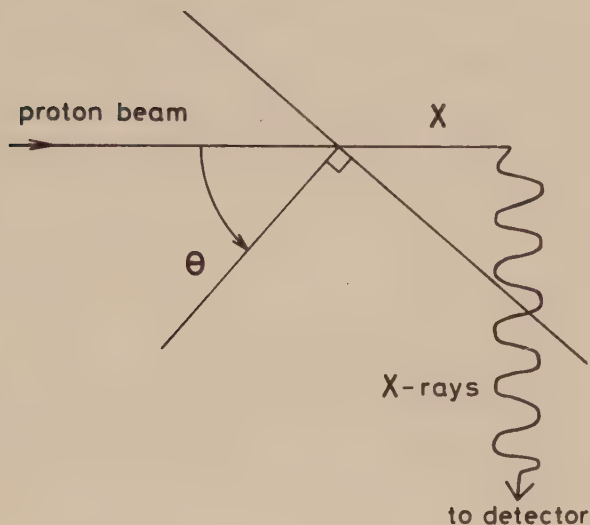


Figure 1.

The orientation of the sample and detector with respect to the beam direction.

With the X-ray detector mounted at 90° with respect to the beam direction, according to Figure 1, the X-ray yield, Y , from an element homogeneously distributed within the sample with a concentration of C atoms per cm^3 is

$$Y = \Sigma/n = k_1 C \int_{E_0}^{E_1} \sigma(E) T(\xi) (-dE/dx)^{-1} dE \quad (1)$$

Σ is the number of pulses from the element when the sample has been irradiated with a proton charge corresponding to n protons. k_1 is a constant including physical parameters which takes into account the detector properties and other experimental parameters

with the exception of σ , which is the ionization cross section. $T(\xi) = \exp(-\mu_m \xi)$ is the transmission of X-rays through the sample (μ_m is the mass absorption coefficient and $\xi = x \rho \cot \theta$ the distance in the sample the X-rays have to pass through, ρ is the density), $-dE/dx$ is the stopping power of the matrix, E_0 and E_1 the initial and final proton energies, respectively.

When the attenuation of X-rays and the decrease in X-ray production cross section due to the slowing down of the protons in the sample can be neglected, which is the case when the element is located as a thin surface layer, the X-ray yield from a concentration of N atoms per cm^2 of the element is

$$Y = \Sigma/n = k_1 \sigma(E_0) N \quad (2)$$

Equations 1 and 2 are fully discussed in reference (4). The equation system for solving for both N and C for any particular element can be written in matrix form

$$\begin{pmatrix} Y_1 \\ Y_2 \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix} \begin{pmatrix} C \\ N \end{pmatrix} \quad (3)$$

The increase in the β/α -ratio for an element homogeneously distributed within the sample matrix as percentage of the ratio from the same element situated as a thin surface layer on to the sample has been calculated for elements with atomic numbers $Z = 15 - 92$. The results are shown in Figure 2 for K X-rays above 2 keV and for L X-rays above 3 keV for the elements distributed in carbon, aluminum, and calcium matrices, assuming an initial proton energy, E_0 , of 2.5 MeV and a target angle, θ , of 45° .

The increase in the β/α -ratio is greater the heavier the matrix element. This is partly due to the lower stopping power of the matrix

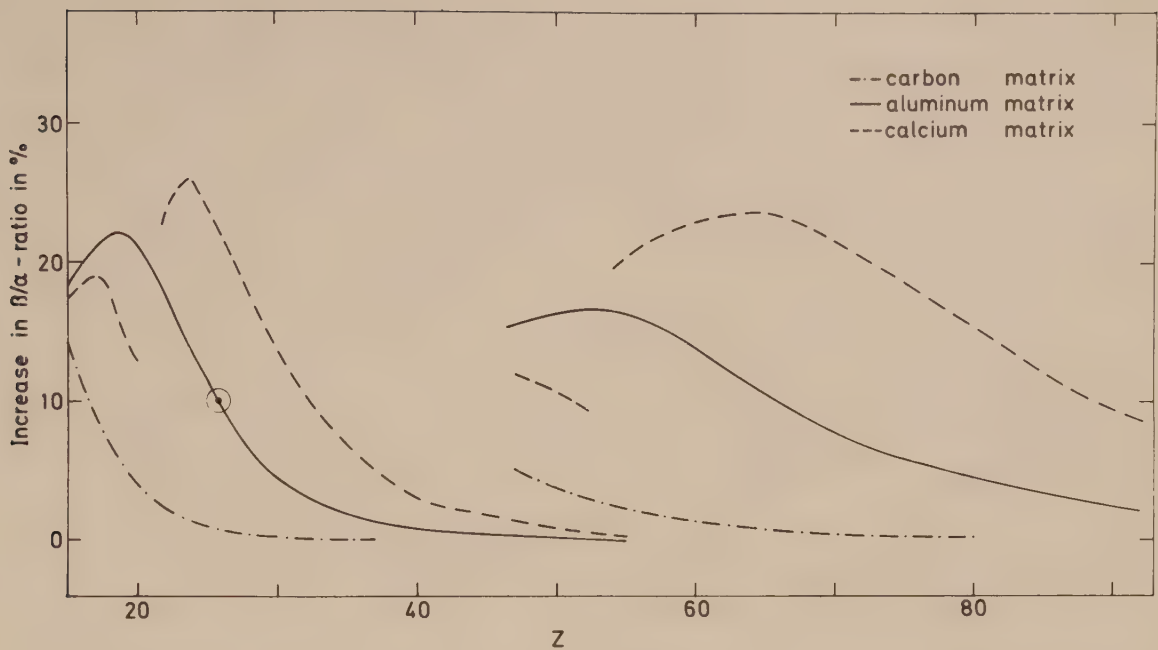


Figure 2. The increase in the K_{β}/K_{α} -ratio for the elements with atomic numbers $Z = 15 - 55$ and in the L_{β}/L_{α} -ratio for the elements with atomic numbers $Z = 47 - 92$, when distributed in different matrices, as percentage of the normal ratio, assuming an initial proton energy of 2.5 MeV and a target angle of 45° . The value for iron in an aluminum matrix is marked out.

which causes the X-rays to be created comparatively deeper in the sample, thus giving them a longer path to the surface, and partly to the higher mass absorption coefficient of the matrix which increases the difference in transmission between the α and β X-rays.

The greater the increase in the β/α -ratio, the more accurate the determination of the distribution will be. A higher proton energy will increase the β/α -ratio for the same reason as the lower stopping power. A decrease in θ will also increase the β/α -ratio by increasing the distance the X-rays have to go in the sample and hence the difference in transmission between the α and β X-rays. The dependence on proton energy and target angle is shown in Figure 3 for iron in an aluminum matrix.

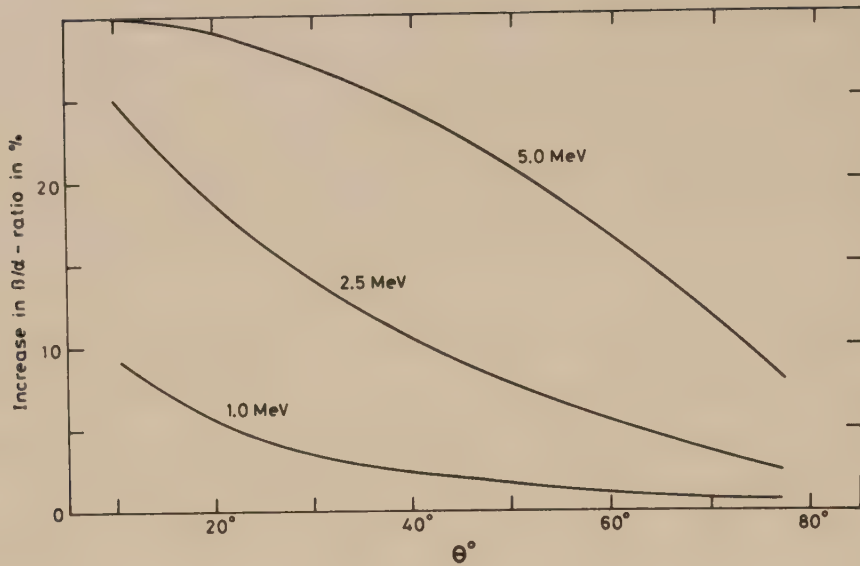


Figure 3. The increase in the K_{β}/K_{α} -ratio for iron in an aluminum matrix as a function of target angle for different proton energies.

An aluminum sample with iron as its main impurity and with a very flat surface was used to test the precision in the determinations of surface concentration N and sample concentration C by the three methods described above.

On to the aluminum, which was found to contain 6 000 ppm iron, $32 \mu\text{g}/\text{cm}^2$ of iron was evaporated. The values were obtained by PIXE-analysis before and after evaporation. For 2.5 MeV protons the yield from the surface layer was estimated to be 1.5 times that from the iron in the aluminum matrix. The sample was then irradiated with 1.0 and 2.5 MeV protons at target angles, θ , of 45° and 22.5° . In Table 1, the values of N and C obtained by combining the irradiations with two proton energies, two target angles and by measuring the β/α -ratio are shown.

The uncertainty in the distribution among the surface and sample matrix (N/C) due to estimated errors in target angle, proton energy, number of pulses, and number of protons are given for each para-

meter in Table 1. It is seen that the precision in the determination of N and C by measuring the β/α -ratio in a single run is competitive with that obtained by using two different proton energies. This is mainly due to the fact that errors in the determination of the charge and hence the number of protons does not affect the β/α -ratio.

Proton energy (MeV)	Target angle (deg.)	X-ray line	N ($\mu\text{g}/\text{cm}^2$)	C (ppm)	Uncertainties in the distribution N/C in % for each parameter error			
					$\Delta\theta=0.5^\circ$	$\Delta E=2\text{keV}$	$\frac{\Delta\Sigma}{\Sigma}=0.5\%$	$\frac{\Delta n}{n}=2\%$
2.5	45	$K_\alpha; K_\beta$	33	5 700	3	0.5	20	0
2.5	22.5	$K_\alpha; K_\beta$	31	6 400	1.5	0.5	10	0
2.5	22.5;45	K_α	29	6 900	40	9	11	40
1.0;2.5	45	K_α	33	5 900	6	3	2	8
1.0;2.5	22.5	K_α	31	6 200	6	4	2	8

Table 1. The concentration of iron on the surface of (N) and in (C) the aluminum sample, determined by measuring the K_β/K_α -ratio, by varying the target angle and by varying the initial proton energy, together with the uncertainty in the distribution N/C due to estimated errors in target angle θ , proton energy E , number of pulses Σ and number of protons n .

From Table 1 it is also seen that the method of measuring the β/α -ratio is very sensitive to errors in the number of pulses in the peaks. This sensitivity can be understood by studying the condition of the matrix A in Equation 3. For an ill-conditioned matrix the determinant is close to 0 and implies a great sensitivity to changes in Y and hence in Σ . As a test for ill-conditioning, the determinant of the normalized matrix

$$a_{ij} \rightarrow \alpha_{ij} = \frac{a_{ij}}{(\sum_j a_{ij}^2)^{1/2}} \quad (4)$$

can be used. If this determinant is small compared to ± 1 , the system is ill-conditioned (12).

When the method of varying the incident proton energy is used, the determinant is 0.5.

Both when measurements at two target angles and of the β/α -ratio are performed, the determinant is about 0.05. This means a great sensitivity to changes in Y and therefore the method of varying the target angle is very uncertain in this investigation due to the errors in the determination of the number of protons, n , and hence to the errors in the X-ray yield, Y .

However, satisfactory results are obtained by measuring the β/α -ratio due to the fact that the α and β X-ray lines are measured simultaneously. This method can therefore be used to determine whether an element is situated on to the surface of the sample or distributed within it, in one single irradiation.

The author acknowledges discussions with Dr R Akselsson and Dr T B Johansson.

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PROTON ACTIVATION ANALYSIS STUDIES ON FLUORINE AND NITROGEN

CONTENT OF DEVELOPING DENTAL ENAMEL

Mats Ahlberg*, James W Bawden**, Lars E Hammarström***
and Ragnar Hellborg*

Departments of Nuclear Physics and Oral Histopathology
University of Lund, Lund, Sweden

ABSTRACT

Proton activation analysis, using the reactions $^{19}\text{F}(p,\alpha\gamma)^{16}\text{O}$ and $^{15}\text{N}(p,\alpha\gamma)^{12}\text{C}$, was employed to study the fluorine and nitrogen content of developing rat molar enamel at various stages of maturation. Four groups of rats were utilized: Group I - injected intraperitoneally at 6 days of age with 20 mg F/kg b w as sodium fluoride solution and terminated at 30 minutes and 1, 5 and 9 days postinjection; Group II - injected in a similar way at 7 days of age and terminated 30 minutes and 1, 4 and 8 days postinjection; Group III - injected with 10 mg F/kg, at 6, 15 and 19 days of age and terminated 30 minutes later; Group IV - control animals injected with distilled water at 6 and 15 day of age respectively and terminated 30 minutes later.

Analysis reflected decreasing nitrogen content in the enamel as maturation proceeded. However, fluorine concentrations were highest in all groups at the earlier stages of enamel development.

* Department of Nuclear Physics, University of Lund
Sölvegatan 14, S-223 62 LUND, Sweden

** School of Dentistry, University of North Carolina,
Chapel Hill, N C 275 14, USA

*** School of Dentistry, S-214 21 Malmö, Sweden

INTRODUCTION

It is well documented that fluorine has important effects on tooth enamel (Fluorides and Human Health, WHO, Geneva, 1970). Numerous investigations have been conducted on the concentration of the element in mature enamel (Brudevold et al, 1956), but little attention has been directed to the concentration of fluorine at various stages of enamel development.

An autoradiographic study by Hammarström (1971) showed that, following an intraperitoneal injection of sodium fluoride ^{18}F in rats 8 to 10 days of age, the most intense localization of ^{18}F in the developing molars occurred in areas of enamel matrix formation, and little or no uptake was seen in areas undergoing final mineralization. Weatherell (1975) has demonstrated that, in the rat incisor, concentrations of fluorine are higher in newly deposited enamel matrix than in more mature mineralized enamel. These results suggest that fluorine may have an important role in the earliest stages of enamel development in association with the organic matrix. Thus, the time sequence of fluorine concentrations in developing enamel, particularly in relation to the protein content, is of interest. The physical half-life of ^{18}F is too short (110 minutes) to permit the desired studies of localization over the appropriate time period. In addition, the high energy radiation emitted by ^{18}F does not allow an optimum degree of geometric resolution when used with standard autoradiographic techniques.

In recent years methods of trace elements analysis using charged particles have developed rapidly. For heavy elements (with atomic number $Z \geq 15$) the analysis is preferably performed by detecting induced characteristic X-rays (Johansson et al, 1970-1972). This method has

been applied to teeth by Ahlberg et al (1975). For lighter elements the weak characteristic X-rays are strongly absorbed. In this case, however, the Coulomb barrier is low and, hence, nuclear reactions with low energy protons are possible. Due to the large Q-value these reactions often result in charged particles and strongly penetrating gamma rays of high energies. A survey of charged particle activation analysis is found, for example, in Amsel et al (1971).

Contrary to X-ray excitation methods, nuclear reactions often exhibit sharp resonances which allow the depth distribution of elements to be rather precisely determined. Studies of this kind have been performed recently on the depth distribution of carbon in steel samples by using the reaction $^{12}\text{C}(\text{p},\gamma)^{13}\text{N}$ (Brune and Hellborg, 1970) and on the depth distribution of fluorine in zircaloy by use of the reaction $^{19}\text{F}(\text{p},\alpha\gamma)^{16}\text{O}$ (Möller and Starfelt, 1967). The latter reaction has also been used recently to study the fluorine concentrations in fully mineralized human enamel (Mandler et al, 1973, Porte et al, 1973, Rytömaa et al, 1974). The purpose of the present study was to use the reaction $^{19}\text{F}(\text{p},\alpha\gamma)^{16}\text{O}$ to investigate fluorine content and distribution at various stages of enamel development in rat molar teeth. In addition, it was possible to use the resonant reaction $^{15}\text{N}(\text{p},\alpha\gamma)^{12}\text{C}$ to determine simultaneously the nitrogen concentration and thus permit estimation of the protein content of the tissue.

MATERIAL AND METHODS

Experimental tissue: Young sucking rats were used as experimental animals. Their mothers were fed pellets with a fluoride content of 40-50 ppm and tap water with a fluoride content of 0.2 ppm. The fluoride content of the milk was probably low. Human milk has been found to contain

less than 0.3 ppm fluoride (Zipkin and Babeaux, 1965).

The rat molars are of limited growth. In the first molar enamel matrix formation starts at the age of 3-4 days and is completed at the age of 7-9 days. The final mineralization starts at 7-8 days and is mainly completed at 15-16 days of age (Kurahashi et al, 1968). In the present experiments the uptake and concentration in the enamel of fluoride after a single injection was studied during these two main stages of development.

Four groups of rats were prepared according to the following protocol:

- Group I: Four littermate rats were given 20 mg F/kg of body weight (b w) as sodium fluoride by intraperitoneal injection at 6 days of age. The solution was sodium fluoride in distilled water. The rats were terminated at 30 minutes, 1 day, 5 days and 9 days postinjection respectively.
- Group II: Four littermate rats were given similar intraperitoneal injections at 7 days of age. They were terminated 30 minutes, 1 day, 4 days and 8 days postinjection.
- Group III: Three littermate rats were given single intraperitoneal injections of sodium fluoride (10 mg F/kg b w) at age 6 days, 15 days and 19 days respectively. All animals were sacrificed 30 minutes postinjection.
- Group IV: Two littermate rats were given a single intraperitoneal injection of distilled water at 6 days and 15 days of age respectively. Both animals were terminated 30 minutes postinjection.

All doses were contained in a volume of 0.05 ml. Following termination by ether, the upper first molar teeth were exposed and carefully removed.

The adhering soft tissues, including the cells of the

enamel organ, were removed under a dissecting microscope. Care was taken to avoid disturbing the enamel matrix. The pulp tissue was also removed and the teeth were allowed to air dry for several days. The pulp chambers were then packed with silver amalgam to provide an electrical ground and increase thermal conductivity to prevent the teeth from becoming highly charged and overheated by the proton beam. Each tooth was glued to an individual aluminium plate in a position to permit the proton beam to hit the mesial buccal surface at as nearly a right angle as possible. Both the maxillary first molars were mounted and analysed. One of each was analysed in detail while the other was checked at two or three depths.

Radiation source and measurements: A proton beam, from a Van de Graaff accelerator, with an energy resolution of 1 keV, and collimated to $0.5 \times 0.5 \text{ mm}^2$, was used. To protect the teeth from being destroyed by excessive heat the beam current was limited to less than 200 nA throughout the experiment. The total charge collected was typically $30 \mu\text{C}$.

The gamma-rays arising from the teeth were initially checked with a high resolution Ge(Li)-detector (effective volume 39 cm^3 , resolution 2.8 keV at an energy of 1.33 MeV). As no peaks were found in the pulse-height distribution which could interfere with the gamma-rays from the nuclear reaction $(p, \alpha\gamma)$ initiated in ^{19}F and ^{15}N , it was possible to use a $5" \times 5"$ NaI(Tl) scintillation detector, which gave about a 30 times higher counting rate than the Ge(Li)-detector. The pulses from the detector were amplified in conventional electronics and stored in a ND-812 multichannel analyser. The experimental arrangement is described by Hellborg et al (1975).

The nuclear reactions used are resonant i.e. they occur at

unique proton energies and, as charged particles loose a well-defined amount of energy per unit path-length, a rather exact localization of the reacting atoms is possible. The measurements were performed at five different proton energies corresponding to different depths in the samples.

The fluorine concentration was determined by the only stable isotope ^{19}F . In the reaction $^{19}\text{F}(\text{p},\alpha\gamma)^{16}\text{O}$ the two resonances at 872 keV and 935 keV with cross-sections of 540 mb and 180 mb respectively, were used (Ajzenberg-Selove, 1972). Another resonance at 1348 keV limits the useful penetration to $2\,500\,\mu\text{g}/\text{cm}^2$. This corresponds to around 28 μm in the 6, 7, 8 and 11 day teeth, and 8 μm in the 15 and 19 day teeth. The difference results from higher densities in the more mineralized enamel.

The nitrogen concentration was determined by the isotope ^{15}N with a relative abundance of 0.37%. Suitable resonant reactions are lacking for the other stable isotope ^{14}N . For the reaction $^{15}\text{N}(\text{p},\alpha\gamma)^{12}\text{C}$ the resonance at proton energy of 897 keV with a cross-section of 1000 mb was used (Ajzenberg-Selove, 1971). Another resonance at 1210 keV limits the useful penetration to $1750\,\mu\text{g}/\text{cm}^2$ (around 18 μm and 6 μm).

The width of 2 keV for the resonance at 897 keV in nitrogen, together with the energy spread of the beam, gave a depth resolution for detecting nitrogen of about $12\,\mu\text{g}/\text{cm}^2$ (1400 Å and 400 Å) close to the surface. Correspondingly, the width of 4.7 keV for the resonance at 872 keV in fluorine, and the energy spread of the beam, gave a depth resolution for detecting fluorine of about $23\,\mu\text{g}/\text{cm}^2$ (2600 Å and 800 Å) close to the surface. For fluorine the investigated zone is broadened to about $400\,\mu\text{g}/\text{cm}^2$ (44 000 Å and 14 000 Å) deeper in

the material, where both resonances take part. Due to straggling, i.e. the statistical fluctuations in the slowing down of the protons in the material, protons starting with equal energies attain the resonant energy at different depths. Thus, in deeper layers the depth resolution is not only determined by the resonance width but also by the straggling. From the calculations of stopping-power and straggling, figure 1 has been constructed showing depth and width of the investigated zones in apatite as a function of initial proton energy.

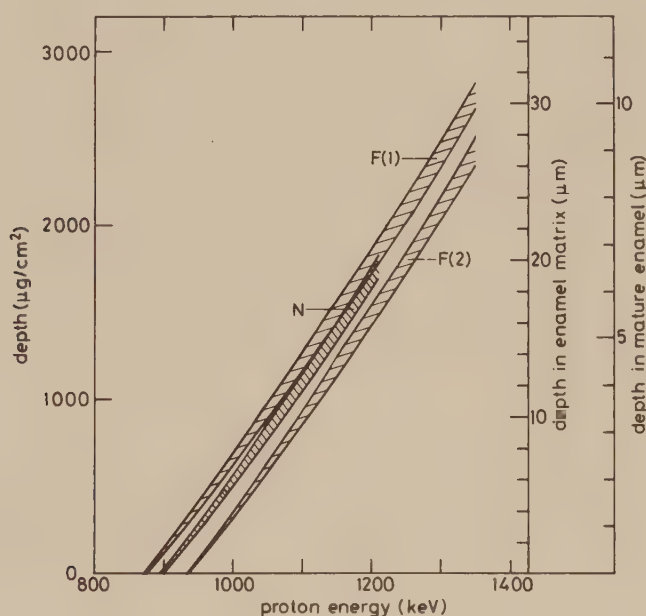


FIGURE 1 The depth and width of the investigated zones in apatite as a function of proton beam energy.

The depth is given in $\mu\text{g}/\text{cm}^2$ and in μm for the enamel matrix and the fully mineralized enamel.

The penetration depths have been calculated using an enamel matrix density of $1.5 \text{ g}/\text{cm}^3$ and using a mature enamel density of $2.8 \text{ g}/\text{cm}^3$ (Deakins, 1942). The nitrogen concentration obtained is related to the zone denoted N and the fluorine concentration obtained is related to the zones denoted F(1) and F(2).

Calcium fluoride (CaF_2) and gallium nitride (GaN) were used as reference standards with known amounts of fluorine and nitrogen. A piece of whale dentine with a fluorine

content of 570 ± 20 ppm as determined by the fluoride electrode technique was used to check the accuracy of the method. (The whale dentin was kindly put at our disposal by professor G Frostell and Dr L G Pettersson, Dept of Cariology, University of Lund.)

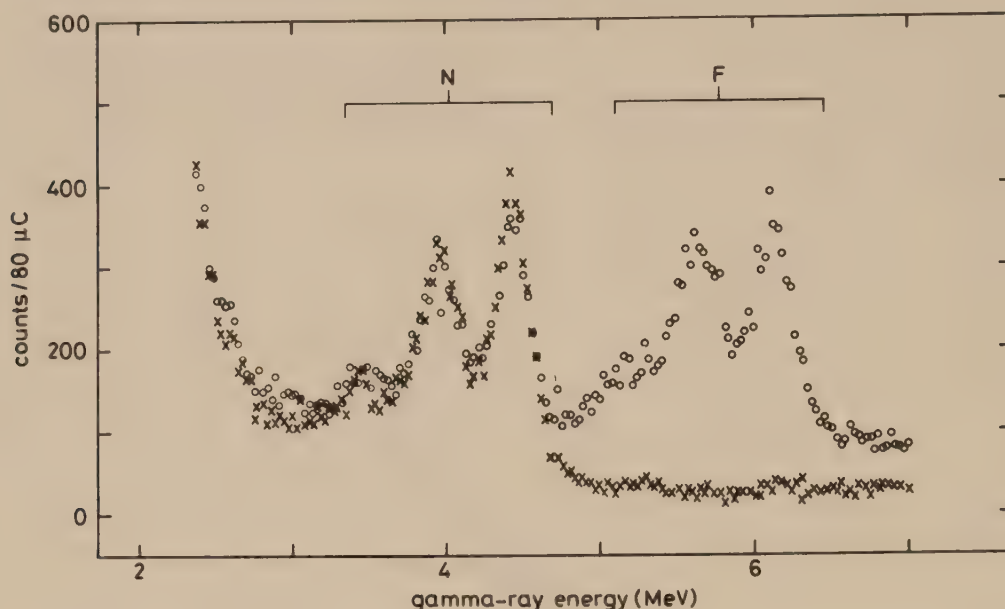


FIGURE 2 Pulse-height distributions from the detector, (O) sample containing 5.1% nitrogen and 185 ppm fluorine, (X) sample containing 7.1% nitrogen and 5 ppm fluorine

Data treatment: Figure 2 shows typical pulse-height spectra. The peak-areas are determined by the computer program SAMPO (Routti and Prussin, 1969) and taken to be proportional to the amount of fluorine and nitrogen in the zone investigated. To derive absolute values, the yield from the teeth has to be normalized to the yield from the reference standards using the relationship that the yield ratio is inversely proportional to the ratio of the stopping-power values.

For protons in CaF_2 , the stopping power values have been taken from Gorodetzky et al (1967). For GaN , apatite and

apatite containing 20-35 per cent protein the stopping-power for protons has been calculated from computed values (Williamson et al, 1966).

The protein has been assumed to consist of approximately 50% C, 25% O, 15% N, 7% H and 1.5% P and 1.5% S (Holleman, 1922). Small variations in these values, as they might relate specifically to the enamel proteins, are not critical to the calculations. As the stopping-power of CaF_2 , apatite, and apatite containing up to 35 per cent protein were found to be equal within 14%, corrections have been made relative only to GaN.

The composition and density of the enamel at various stages of formation have been obtained from Deakins (1942).

According to him the early enamel matrix has a density of 1.5 g/cm^3 and contains 37% inorganic material, 19% organic material, and 44% water. The fully mineralized enamel has a density of 2.8 g/cm^3 and contains 95% inorganic material, 1.8% organic material, and 4.3% water. All water in the tissue samples was removed by air drying and subsequent exposure to high vacuum during irradiation.

The density and composition for the 11 day and younger samples have been assumed to be that of enamel matrix. Therefore, the nitrogen and fluorine values obtained, as well as the depth readings were adjusted to account for the water loss in order to accurately estimate the situation in vivo. The 15 day and older samples were assumed to be fully mineralized and no such adjustment was necessary.

RESULTS

Proton activation analysis of the whale dentin gave a fluorine content of $540 \text{ ppm} \pm 50$. The values were obtained from 4 different analysis on two samples. This value compares favourably with values obtained by a fluoride electrode method ($570 \text{ ppm} \pm 20$).

In the rats teeth the concentration of fluorine and nitrogen at the various depths and at the various ages of the rats are given in tables I and II. The results from the two upper first molars from the same rats were identical, within statistical errors, regarding both nitrogen and fluorine content.

TABLE I Fluorine concentration in the first upper molar of young rats. The figures have been calculated to give the concentration (ppm by weight) in vivo and also to give the depth gradient in vivo. Thus it has been taken into account in the teeth from 6-11-day-old rats that the water content (40% by weight according to Deakins, 1942) of the enamel matrix had been lost prior to the activation analysis.

Experimental group I : Rats injected with 20 mg F/kg b w at 6 days of age and terminated 30 minutes, 1 day, 5 days and 9 days postinjection.

Experimental group II: Rats injected with 20 mg F/kg b w at 7 days of age and terminated 30 minutes, 1 day, 4 days and 8 days postinjection.

Experimental group III: Rats injected with 10 mg F/kg b w at age 6 days, 15 days and 19 days respectively and terminated 30 minutes postinjection.

Experimental group IV: Rats injected with distilled water at 6 days and 15 days of age respectively and terminated 30 minutes postinjection.

Exper. group	Depth (μ m)	D a y s o f a g e					
		6	7	8	11	15	19
Gr I	1.1					50	
	2.3	230	660		88	42	
	4.6	200	450		74	35	
	8.4	83	310		42	48	
	15.8	88	250		25		
Gr II	1.1					95	
	2.3		130	270	58	65	
	4.6		120	250	54	89	
	8.4		88	140	49	110	
	15.8		79	130	68		
Gr III	1.1					71	37
	2.3	130				<10	24
	4.6	130				<10	26
	8.4	86					
	15.8	91					
Gr IV	1.1					14	
	2.3	36				13	
	4.6	20				10	
	8.4	9					
	15.8	8					

TABLE II Nitrogen concentration in the first upper molar of young rats. The figures have been adjusted in the same way as in table I to give the concentration (% by weight) and depth gradient in vivo. For the description of the experimental groups see the text to table I.

Exper. group	Depth (μm)	D a y s o f a g e					
		6	7	8	11	15	19
Gr I	0.4					3.2	
	2.5	5.1	2.5		2.6	2.7	
	4.6	6.8	3.5		2.9	2.3	
	8.8	4.7	3.6		3.5		
	15.8	5.5	3.2				
Gr II	0.4					2.8	
	2.5		2.3	3.4	2.6	1.7	
	4.6		4.1	2.9	2.4	2.2	
	8.8		2.7	2.5	2.6		
	15.8		3.8	2.5	2.5		
Gr III	0.4					1.2	≤ 0.5
	2.5	3.2				1.0	≤ 0.3
	4.6	2.7				0.8	≤ 0.3
	8.8	1.4					
	15.8	1.6					
Gr IV	0.4					1.1	
	2.5	2.1				0.9	
	4.6	1.1				1.0	
	8.8	1.7					
	15.8	1.3					

The fluorine concentration was usually highest at the surface with a decrease in the deeper layers. This gradient was less in the more mineralized enamel.

In animals injected with fluoride at the age of 6 or 7 days the concentration of fluorine in the surface enamel at 6, 7, and 8 days was higher than at 11 and 15 days. The same relationship was found in the animals killed 30 minutes postinjection (group III) and in those animals which had received their fluoride in the diet (group IV). The values at a depth of 2.3 μm for groups I and II are shown in figure 3a.

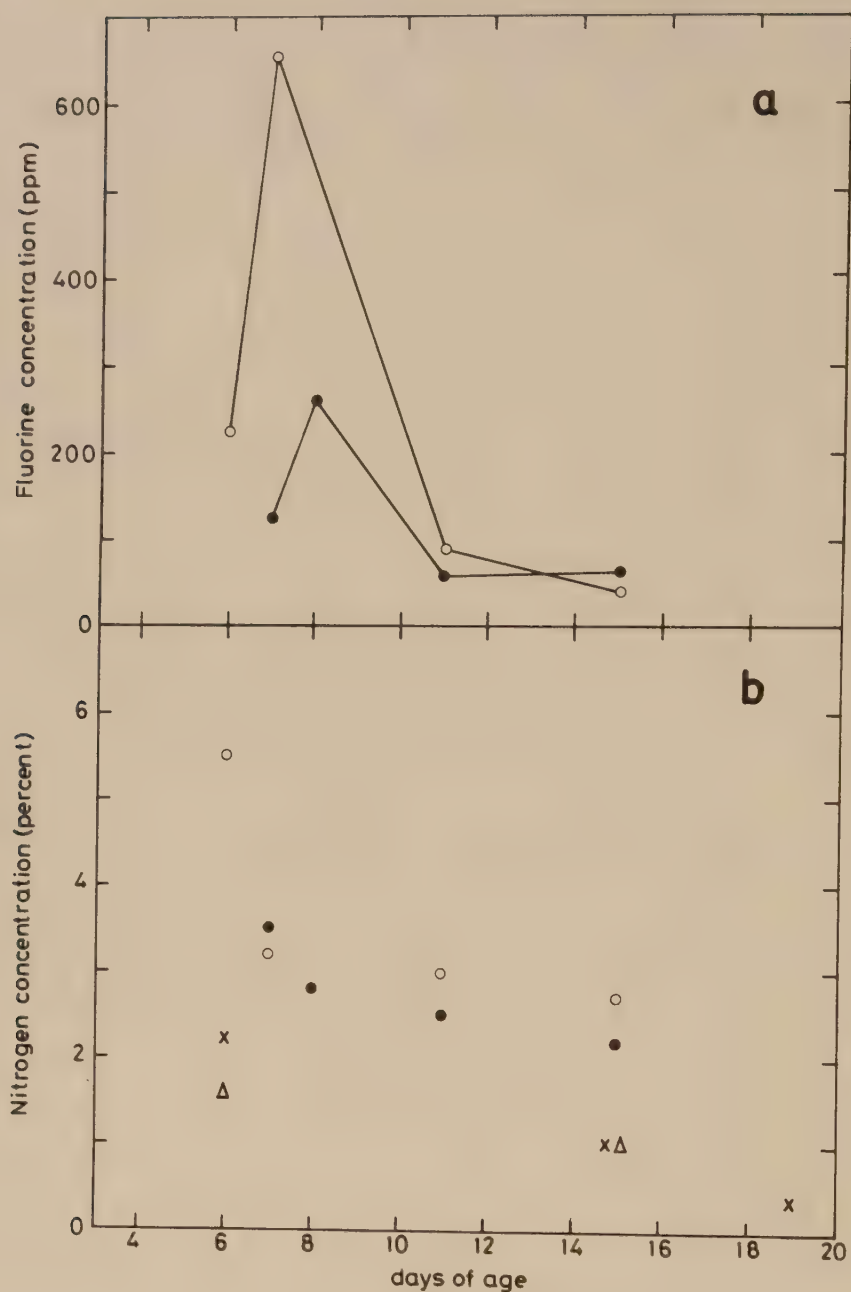


FIGURE 3 The fluorine (a) and nitrogen (b) concentration as a function of age for the different preparation groups

(O) - group I (●) - group II
 (X) - group III (Δ) - group IV

The fluorine values are for a depth of 2.3 microns in vivo and nitrogen values are pooled from the various depths studied. For the description of the experimental groups see "Materials and Methods" and the text to table I.

The nitrogen values were about 3% in the enamel matrix in the younger rats and less than 0.5% in the fully mineralized enamel of the 19 day old rat. There seemed to be more nitrogen left in the enamel when the animals were injected with fluoride during the stage of matrix formation than in animals not injected with fluoride, or injected in later stages of tooth development (Table II). There was no demonstrable depth gradient. The values from each animal were pooled and are shown in Figure 3b.

DISCUSSION

The resonances of the two nuclear reactions used in this study make it possible to determine the concentrations of fluorine and nitrogen at the same depth in a single sample in one measurement. This can be seen in figure 1 which shows the depth and width of the investigated zone as a function of initial proton energy. By such an approach one can relate fluorine content with various stages of enamel maturation.

The sensitivity of the method was illustrated in one sample (not shown in the tables) where it was possible to detect a fluorine concentration of only 2 ppm when the charge collected was raised to 400 μC . This concentration, together with a beam area of $0.5 \times 0.5 \text{ mm}^2$ and a resonance width of $\Gamma = 4.7 \text{ keV}$ (for the 872 keV resonance), gave a calculated fluorine quantity of $2 \times 10^{-13} \text{ g}$.

There are some conditions which must be considered when the method is applied as it was in this study. One problem relates to the small size of the rat molar teeth and the fact that the minimum practical dimension of the proton beam at the target surface was 0.25 mm^2 . The beam had to be precisely aligned to be certain that all of the beam struck the surface of the tooth, and that none of it went

by the target. By observation of the beam during irradiation, and the location of the blackened area of the tooth surface following analysis, it appeared that all of the beam struck the tooth surface in each instance. However, it is possible that some error of this type occurred.

While the largest relatively flat area of the tooth surface was selected as the target area (the mesial-buccal surface), it was not completely flat. Thus, the beam did not strike the surface at a right angle in the entirety of the target area, and the depth of the analysed zone measured perpendicular to the surface was slightly variable. In addition, the surface of the enamel in the matrix stage became roughened or uneven when the water was lost. Therefore, depth readings must be regarded as approximate.

Particularly at 6, 7, and 8 days is the rat first molar tooth in a dynamic stage of development. The cuspal areas are in the postsecretory stage while matrix deposition is still occurring in the more cervical regions (Kurahashi et al, 1968). At 11 and 15 days the differences are not so great even though mineralization in the caspal areas is more advanced. Since the proton beam area covered nearly the entire surface in the cervical-to-cusp dimension, it must be emphasized that the recorded values for nitrogen and fluorine should be regarded as mean values for the surface at the particular day of development.

Extreme care was taken during removal of the teeth from the animal and removal of the cells from the enamel surface. As far as could be determined, the enamel surface was not disturbed, but the possibility of such an error cannot be entirely dismissed.

The different matrices give an error of 14% due to different stopping power values. Peak fitting by the program SAMPO had an error of 7%. Since these are measured

values, they are treated quadratically to give a combined error of 16%. The estimated maximum error of 5% in charge measurement is then added, resulting in a total calculated error of approximately 20%.

For the whale dentin standard the error due to different stopping power values is about half that of the rat teeth. Due to better statistics the error in the peak fitting is decreased to 5%. The quadratic combination of these errors is 9%. Together with the 5% error in the charge measurement the total error for the whale dentin standard is approximately 15%. Repeated measurements on the standards indicated that the error was about 10%.

In all groups of animals the nitrogen values followed the expected pattern of decreasing concentration with advancing age, reflecting loss of protein from the enamel matrix as mineralization proceeds. Conversion of the per cent nitrogen to estimated protein content gave values in general agreement with other investigators, both when measured in dried samples and with correction for water loss (Deakins, 1942). Of specific interest was the finding of an increased nitrogen retention in the mineralizing enamel in animals which had a higher fluorine content in the enamel matrix. Even though the number of teeth is too small to draw any definite conclusions, the data suggest that the fluoride in the enamel matrix in some way delays or inhibits the maturation process. Studies on human teeth have shown an increased nitrogen content in fluorosed teeth compared with teeth which showed no evidence of fluorosis (Bhussry, 1958). The histopathological features of fluorosed human enamel examined by polarized light and by microradiography are similar to those seen in developing enamel, which indicates that the maturation process is disturbed by a high concentration of fluoride ions (Fejerskov et al, 1975).

Of most interest were the Group II animals. The final deposition of enamel matrix occurred over a major portion of the surface between the time of injection of fluoride and the time of sacrifice 24 hours later (Deakins, 1942). Thus, analysis of corresponding layers of surface enamel should have occurred in this group at 8, 11, and 15 days. The data indicate that the fluorine concentration was higher at day 8 than at days 11 and 15. It would seem that relatively high amounts of fluorine are retained in the enamel at the time of, or shortly after, matrix deposition. As calcification proceeds and organic content is lost, some of the fluorine is also removed. These findings are in accord with the work of Weatherell et al (1975).

In the group I animals, it would be expected that the high fluorine value observed at day 7 would be found in a layer at a depth beyond the penetration of the beam at 11 and 15 days (Zellman and Hammarström, Personal communication).

Fluorine levels in the group III animals indicate that diffusion of fluorine into the enamel is more rapid at 6 days than at 15 days. This is in agreement with previous work reported by Hammarström (1971).

While values were low in the control animals (Group IV), the fluorine concentration was higher at 6 days than at 15. The diet was the apparent source of fluorine.

It would appear, based on the present study and other findings (Weatherell, 1975), that fluorine accumulates in relatively high concentrations in early enamel matrix formation, possibly reflecting the high water content of the tissue at that stage of development and ease of diffusion of the ion into area. The fluorine seems to be reduced in concentration as mineralization proceeds.

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